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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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THE RELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE IN SEGREGATES OF PARTIALLY STERILE WHEAT HYBRIDS[1]

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Received January 19, 1923

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INTRODUCTION

Crosses between *Triticum vulgare* with 21 gametic chromosomes and *T. durum* with 14 gametic chromosomes result in partially sterile F_1 plants and F_2 segregates with all degrees of sterility. In the reduction divisions of the F_1 pollen mother cells there are 14 bivalent and 7 univalent chromosomes. The bivalent chromosomes divide normally in both divisions, but the univalent chromosomes divide equationally in the first division after the members of the bivalents have passed to the poles, and in the second division the univalent chromosomes pass at random to one pole or the other without dividing. Thus the F_1 gametes contain from 14 to 21 chromosomes, the frequency distribution depending on random assortment of the 7 univalent chromosomes (SAX 1921, 1922).

Sterility in the F_1 and later generations can be attributed to the unbalanced numerical relations of the chromosomes, due to the random distribution of the univalents, and to incompatibility of certain chromosome combinations even among the bivalents. It is assumed that gametic and somatic perfection increase as the chromosome number and constitution approach the parental types. The seven additional chromosomes of *T. vulgare* apparently determine the characters which distinguish the *vulgare* from the *durum* and other 14-chromosome wheats. The above

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conclusions in regard to sterility will explain the partial association of parental characters in F_2 segregates, the occurrence of species of Triticum with chromosome numbers in multiples of seven, and the difficulty in obtaining homozygous segregates combining the desirable characters of the Vulgare and Emmer wheats. Cytological and genetic data are now available which support the above conclusions and show clearly the relation between chromosome number, morphological characters and rust resistance.

STERILITY IN HYBRIDS OF *Triticum vulgare* × *T. durum*

Sterility in wheat hybrids can be measured by grains set per spikelet and by the percentage of obviously poor pollen grains. The amount sterility varies in different species crosses and even in the same species cross with different varieties (SAX 1921). In the F_1 hybrids between *T. vulgare* and *T. durum* the degree of sterility as measured by grains set per spikelet varied from 50 to 83 percent. From 11 to 21 percent of the F_1 pollen grains were obviously aborted as compared with about 1 percent for the parents, but many of the apparently morphologically perfect F_1 pollen grains were probably not functional. It is assumed that all of the fertile egg cells are fertilized because of the large number of pollen grains available in each flower.[2] Some of these F_1 pollen grains are functional and it is probable that there is selective fertilization if gametic perfection increases as the chromosome number approaches 14 or 21.

Since sterility is measured by both male and female gametic development it is of interest to determine to what extent the same factors are involved. Considerable correlation was found in the F_3 segregates of Amby × Kubanka between the percent of imperfect pollen and the number of grains per spikelet ($r = -.49 \pm .07$). One might expect a higher correlation if sterility of pollen grains and egg cells is due to the same causes. However, many apparently morphologically perfect pollen grains may not be functional and the absence of grain development of the plant may be due to weak somatic development and not due to gametic sterility. Considering the various factors which may cause variability in gametic sterility the correlation obtained may indicate that the same factors are involved in the sterility of egg cells and pollen grains.

[2] If fertilization is not effected by the pollen from the same flower the glumes remain open a long time thus permitting cross-pollination instead of the usual self-pollination. It is probable that cross-pollination occurs more frequently in partially sterile hybrids than in completely fertile individuals, but the amount of cross-pollination which occurs would not greatly affect the general cytological and genetic correlations found in such hybrids. The increased susceptibility to ergot which BIFFEN (1912) finds in partially sterile hybrids is undoubtedly due to the long period during which many of the flowers remain open and exposed to the spores of this fungus.

The degree of sterility in the F_2 and F_3 has been obtained for the cross *T. vulgare* (Amby) × *T. durum* (Kubanka). The frequency distribution of sterility as indicated by grains set per spikelet are shown in table 1. In the F_2 and F_3 segregates only the grains set in the outer florets of each spikelet were counted so that 1.8 to 2.0 grains per spikelet would represent the fertility of the parents. The degree of sterility in this particular cross is about 80 percent in F_1, 50 percent in F_2 and 25 percent in F_3. It is evident that the degree of sterility rapidly decreases in successive generations even though much of the sterility in F_2 and F_3 is due to weak somatic development as well as gametic imperfections.

TABLE 1

Sterility in F_2 and F_3 of T. vulgare × T. durum.

Grains per spikelet.

	0-.2	.2	.4	.6	.8	1.0	1.2	1.4	1.6	1.8	2.0-2.2	*n*	Mean	S. D.
F_2 frequency...	7	6	4	3	4	6	1	5	4	9	3	52	1.07± .06	.68± .04
F_3 frequency...			2		2	1	2	9	8	12	2	38	1.59± .04	.38± .03

It has been assumed that sterility is due to unbalanced numerical relationships of the chromosomes as a result of random assortment of the 7 univalents, and to incompatibility of certain chromosome combinations even among the bivalents (SAX 1922). If all gametes with 17 or 18 chromosomes rarely function, then the degree of sterility found in F_1 individuals would be largely accounted for. On this basis 70 gametes in a total of 128 would be eliminated. It is probable that gametes with 16 and 19 chromosomes are also less fertile than those with 21 or 14 chromosomes. The frequency distributions of all combinations of univalents and for the 14 bivalents are shown in table 2. It is evident from this table that only 1 gamete in 64 will have either 14 or 21 chromosomes, i.e., either 0 or 7 univalents.

TABLE 2

Recombinations of Emmer and Vulgare chromosomes in F_1 gametes. Frequencies given for the 7 singles and the 14 members of bivalents.

Vulgare.....	0	1	2	3	4	5	6	7							
Frequencies.....	1	7	21	35	35	21	7	1							
Emmer.....	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
Vulgare.....	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Frequencies.....	1	14	91	364	1001	2002	3003	3432	3003	2002	1001	364	91	14	1

It is also clear that unless there is almost complete compatibility among the 14 members of the bivalents, considerable sterility would result. For instance, if combinations of equal numbers of *durum* and *vulgare* chromosomes or 6 chromosomes from one parent and 8 chromosomes from the other parent resulted in non-functional gametes, then 9,438 or 58 percent of the bivalent combinations would be sterile. Evidence to be presented later indicates that most of the sterility can be attributed to the irregular behavior of the 7 univalent chromosomes, so it must be assumed that, with few exceptions, all combinations of *vulgare* and *durum* chromosomes are compatible among the 14 primary chromosomes in the F_1 gametes.

In the F_2, sterility may be due to unfavorable somatic development or to gametic sterility *per se*. In the F_1, two complete sets of chromosomes are present and the F_1 plants are unusually large and vigorous. In the F_2, however, certain chromosome combinations inhibit vegetative development so that many seeds do not germinate or if they germinate they do not pass the rosette stage. Under greenhouse conditions about half of the F_1 seeds produce F_2 plants which bear grain. Under field conditions this proportion may be considerably less. In a cross of *T. vulgare* × *T. polonicum* 309 F_1 seeds gave 97 F_2 plants in 1922.

If the female gametes with 17 or 18 chromosomes do not develop and those with 16 or 19 chromosomes are less perfect than those with 14 or 21 chromosomes the sterility of the female gametes is largely accounted for. Selective fertilization would be expected among the pollen grains, those with 14 or 21 chromosomes being more perfect would function with greatest frequency. In the F_2 and subsequent generations there is further elimination of intermediate types in both somatic and gametic development. As a result of these various factors three classes of segregates would be expected, one group with the chromosome number similar to that of the *durum* parent, another group with the chromosome number similar to that of the *vulgare* parent and a third group with an intermediate chromosome number.

THE CHROMOSOME NUMBER IN F_2 AND F_3 SEGREGATES OF *T. vulgare* × *T. durum* AND *T. polonicum*

The chromosome counts for F_2 and F_3 plants were determined from preparations made by BELLING'S (1921) method. Pollen mother cells were mounted in iron aceto-carmine and counts were made without pressing out the cell contents. In many cases camera lucida drawings were made. It was often difficult to distinguish univalents from bivalent

chromosomes, especially in the metaphase, so in correlating chromosome number with morphological characters the total haploid chromosome number was used. In several cases however 14 ± 1 bivalents and 5 to 7 univalents could be counted. It was also difficult in many cases to get the chromosome count more accurate than ± 1. In grouping the chromosome counts the few plants with counts of 15 or 20 were classed as 14 or 21, respectively, and all plants with an intermediate chromosome number were grouped. In table 3 the calculated somatic chromosome number is given for 15 F_2 segregates of Red Fife × Polish and for 46 F_3 segregates of Amby × Kubanka.

TABLE 3

Frequency of chromosome numbers in partially sterile wheat hybrids.

HYBRIDS	DIPLOID CHROMOSOME NUMBER															n
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
T. vulgare × *T. polonicum* (F_2)	5				1		1	5	2						1	15
T. vulgare × *T. durum* (F_3)	21		1	1	1	1	1	4	2	1	1				12	46

It is evident that a far greater number of F_2 segregates have either 14 or 21 chromosomes than would be expected if all gametes were functional. With no elimination of gametes and no selective fertilization only 2 plants in 16,384 F_2 segregates would have either 28 or 42 chromosomes. In both F_2 and F_3 segregates the plants with intermediate chromosome numbers could in some cases be attributed to the union of gametes with chromosome numbers similar to the two parental types. The above distributions of chromosome counts show clearly that the functional perfection of the gametes increases as the chromosome number approaches the parental type and that gametes and plants with an intermediate chromosome number are rapidly eliminated.

THE CORRELATION BETWEEN CHROMOSOME NUMBER, MORPHOLOGICAL CHARACTERS AND RUST RESISTANCE

The relations between chromosome number, morphological characters and rust resistance were determined for F_3 segregates of *T. vulgare* × *T. durum* (Amby × Kubanka). Data on rust resistance were obtained in the field. The climatic conditions in 1922 were very favorable for rust development and susceptible varieties were very severely infected. The rust found was *Puccinia graminis* as indicated by the pustules and the shape of the teleutospores, but it was not possible to class it in any of the 38

biologic forms described by STAKMAN and LEVINE (1922). In my cultures Einkorn was very resistant, Kubanka was moderately resistant, at least, while Marquis and a club wheat very similar to Little Club were both extremely susceptible. A scale of rust resistance similar to those used by previous investigators was adopted. Data on pollen-grain size and variability were obtained from measurements of about 60 pollen grains from each F_3 plant. The following code was used in describing the F_3 plants.

CODE FOR DESCRIPTIONS OF WHEAT HYBRIDS

K = Kubanka. A = Amby.

a. Rust resistance
   1. Very resistant but not immune, K
   2 to 5. Intermediate stages
   6. Very susceptible A
b. Mean pollen-grain diameter in microns; A = 60, K = 55
f. General type of head
   1. Vulgare type, A
   2. Intermediate
   3. Emmer type, K
g. Diameter of culm in mm; A = 2.2, K = 1.5
h. Spikelet shape
   1. Open as in Amby
   2. Intermediate
   3. Compressed as in Kubanka
i. Keel hape
   1. Small as in Amby
   2. Intermediate
   3. Large as in Kubanka
j. Length of longest beard in cm; A = 0, K = 17
n. Compactness of head
   1. Compact as in Kubanka
   2. Intermediate
   3. Open as in Amby
o. Chromosome number, (haploid); A = 21, K = 14
q. Standard deviation of pollen-grain diameter
r. Percent of poor pollen
s. Grains per spikelet

In each case the characters of the two parents are indicated. For correlations between different morphological characters, data on 52 plants are available; for the relation between rust resistance and morphological characters 49 plants are described; for the relation between chromosome number and morphological characters 38 plants are described and for correlations between chromosome number and rust resistance 37 plants are available. The different numbers used are largely due to the fact that many plants for which chromosome counts were made had only one head and the latter was used for chromosome counts. Chromosome counts

were obtained for 25 F_3 families. In practically all cases members of the same family,—i.e., descendants of the same F_2 plant,—had the same chromosome number.

The classification of characters into broad categories and the bimodal frequency distribution in many cases made the use of the correlation coefficient, as a measure of association, of doubtful value. Accordingly, for such cases the coefficient of contingency η was used (PEARSON 1913).[3] The probable error of the coefficient of contingency was taken as four-thirds of the probable error of the correlation coefficient for the same value of n and r. In cases where only one side of the correlation table was classed in large groups the correlation ratio, r_c, was used. When both sides of the correlation table were classed in numerical measurements the correlation coefficient was used.

The correlations between chromosome number, morphological characters, and rust resistance are shown in table 4. There is a very high degree of correlation between chromosome number and rust resistance, head type, keel shape, spikelet shape, and head shape. All of these characters are those which distinguish the *durum* and *vulgare* wheats. For instance the *durum* wheats have head type 3, keel shape 3, spikelet shape 3, while the *vulgare* wheats have the first type for each of these characters (see description of characters p. 306). There is not much correlation between awn length and chromosome number, because awned and awnless segregates were found in both 14- and 21-chromosome types. This relation is in accord with taxonomic differences, because both *durum* and *vulgare* wheats may be awned or awnless although the *durum* wheats usually have long awns. It will be noted that all segregates with very long awns, i.e., 12 cm or longer, have 14 chromosomes, while the segregates which are true awnless types have more than 14 chromosomes. This again is in accord with taxonomic differences, as only the *vulgare* wheats have true awnless varieties like Amby, and the awns of the awned types do not exceed 10 cm in length. The awns of *durum* varieties vary from 12 to 23 cm in length and in Kubanka are about 17 cm long. The correlation between chromosome number and culm diameter is not high, because culm diameter varies greatly, depending on the vigor of the plant.

It is evident that all of the distinguishing characters of the *durum* parent are found only in 14-chromosome segregates and that the typical *vulgare* characters are found in the 21-chromosome segregates. In the first correlation table one segregate with 14-chromosomes was classed as a

[3] The writer is indebted to Dr. JOHN W. GOWEN for suggesting and demonstrating the use of this method.

vulgare type, but a detailed classification of the distinguishing characters did not sustain this classification. One 21-chromosome segregate was consistently classed as a *durum* type, but on looking up the original chromosome counts it was found that this plant had 14 ± 1 bivalents and 6 ± 1 univalents, so that it resembled the F_1 in chromosome number and re-

TABLE 4

Showing correlation between chromosome number and morphological and physiological characters in F_3 segregates of a cross of T. vulgare × T. durum.

CHARACTERS CORRELATED			CHROMOSOME NUMBER 14	16–19	21	
f. Head type	(V)	1	1	4	8	r_c
		2	6	2	3	$-.73 \pm .07$
	(E)	3	12	1	1	
i. Keel shape	(V)	1		3	9	r_c
		2	4	3	2	$-.88 \pm .03$
	(E)	3	15	1	1	
h. Spikelet shape	(V)	1		4	9	r_c
		2	5	1		$-.82 \pm .05$
	(E)	3	14	2	3	
n. Head shape	(E)	1	16	1	1	r_c
		2	3	5	4	$.87 \pm .03$
	(V)	3		1	7	
g. Diameter of culm	1.0 to 1.2		1			η
	1.2		4			$.46 \pm .09$
	1.4		6	2		
	1.6		4	1	6	
	1.8		2	1	1	
	2.0		1	2	1	
	2.2		1	1	1	
	2.4 to 2.5				2	
j. Length of beards	0			2	3	η
	0–2		9	2	4	$.15 \pm .11$
	2–4		4		1	
	4–6		2		2	
	6–8			2		
	8–10				1	
	10–12			1	1	
	12–14		2			
	14–16		1			
	16–18		1			

TABLE 4 (continued)

CHARACTERS CORRELATED			RUST RESISTANCE 1	2	3	4	5	6	
o. Chromosome number	(E)	14	2	14	2				r_c
		16–19			2		1	4	.87± .04
	(V)	21		1	1	2	3	5	
f. Head type	(V)	1			3		4	7	r_c
		2		8	1	1	1	3	−.79± .05
	(E)	3	7	11	1	1		1	
i. Keel shape	(V)	1			2	1	4	6	r_c
		2	1	2	1		1	5	−.82± .03
	(E)	3	6	17	2	1			
h. Spikelet shape	(V)	1			2	1	3	7	r_c
		2		4	2		1	3	−.77± .05
	(E)	3	7	15	1	1	1	1	
n. Head shape	(E)	1	7	16	2	1	1		r_c
		2		3	2		2	6	.78± .05
	(V)	3			1	1	2	5	
j. Length of beards	(V)	0					1	4	
		0.2	1	11	1			7	
		2–4	1	3	1		2		
		4–6		2			2		
		6–8	1		2				η
		8–10				1			.59± .06
		10–12		1					
		12–14	1		1	1			
		14–16	2	1					
	(E)	16–18	1	1					

sembled the *durum* parent in most respects. This plant was relatively sterile and had only 0.9 grains per spikelet. Two other segregates classed as 21-chromosome types had at least several lagging chromosomes.

Since these differentiating characters of the parents are all closely associated with chromosome number they should be more or less closely associated with each other. We do find a very high correlation between the various morphological characters. The coefficient of contingency for keel shape and spikelet shape is .99 ± .02, for keel shape and head shape .91 ± .02, and the correlation ratio for keel shape and diameter of culm is −.66 ± .05. In general the shape of the keel alone could be used satisfactorily as a means of classifying segregates into *durum* or *vulgare* types.

There is a striking relation between chromosome number and pollen-grain size in the cultivated species of wheat (SAX 1922). There is also a high correlation between chromosome number and pollen-grain size in the F_2 segregates ($\eta = .77 \pm .05$). The average size of the pollen grains could be used as an indication of the approximate chromosome number in parents or segregates.

It is evident from table 4 that rust resistance is also closely associated with morphological characters. Segregates which have the chromosome number and morphological characters of the resistant *durum* parent are in general resistant to rust while the segregates which have 21 chromosomes and resemble the *vulgare* parent are usually susceptible. Especially interesting is the relation between rust resistance and length of awns. The true awnless segregates are very susceptible, while the segregates with very long awns are resistant to rust. The segregates with short awns which would ordinarily be classed as awnless, may be either susceptible or resistant, but with an increase in awn length resistance increases. The number of segregates with the Vulgare type of awn is too small for conclusive results, but it is perhaps significant that the most important resistant varieties of *T. vulgare*, Kanred and Kota, and other Crimean selections, are all awned.

The association of rust resistance and morphological characters in segregates of *T. vulgare* × *T. durum* has been attributed to chromosome linkage by HAYES, PARKER and KURTZWEIL (1920) and by WALDRON (1921). WALDRON has suggested that the linkage of *durum* head characters and rust resistance in the segregates might be due to the association of these characters in two chromosomes of the same pair and that crossing-over would produce the occasional resistant *vulgare* type which is occasionally found. FREEMAN (1917) describes linkage between shape of head (the same character which is called shape of spikelet in the present paper) and hardness of grain, in F_2 segregates of *T. vulgare* × *T. durum*. In view of the cytological and genetic data here presented it is clear that the association of rust resistance and morphological characters is not due to chromosome linkage in the usual sense of the term, but is due to the fact that most of the surviving segregates have 14 or 21 chromosomes and that rust resistance and distinguishing morphological characters are in general associated with chromosome number.

The fact that chromosome number is so clearly associated with rust resistance and morphological characters in segregates of *T. vulgare* × *T. durum* and in the cultivated species, suggests that the 7 additional chromosomes of the *vulgare* parent determine the distinguishing charac-

ters of the *vulgare* wheats. The effect of the 7 additional chromosomes may be due either to the numerical relations causing a reduplication of hereditary factors, or to specific factors in these chromosomes which are not possessed by the 14 chromosomes of the Emmer wheats. If the latter hypothesis were correct it would be expected that these 7 chromosomes which determine the distinguishing characters of the Vulgare wheats would not pair with the Emmer chromosomes and thus no 14 chromosome segregates would possess the physiological and morphological characters of the Vulgare wheats. In either case it would be impossible to combine the desirable characters of the Emmer and Vulgare wheats in a homozygous fertile segregate.

The genetic data show that the characters which are common to the two groups of species may combine and segregate with the usual Mendelian results. For instance, in a cross of *T. vulgare* × *T. durum*, LOVE and CRAIG (1919) find a 15 : 1 ratio for color of chaff and for color of grain, and a ratio approximating 3 : 1 for the segregation of awnless and awned segregates. The awned condition is associated to some extent with differences in chromosome number, as I have shown, so that a clear case of simple segregation would not be expected. PERCIVAL (1921) finds normal segregation of glume color in a similar cross. The characters found in both groups of species are apparently determined by the 14 primary chromosomes. Since there is in most cases an orderly behavior of these chromosomes normal segregation and recombination would be expected. On the other hand, the characters peculiar to the Vulgare wheats are dependent on the assortment of the 7 univalent chromosomes and as a result these characters, in a homozygous condition, are, with few exceptions, found only in segregates with 21 chromosomes.

In species hybrids which are relatively sterile, such as Amby × Kubanka, the 7 additional chromosomes of the Vulgare parent may be quite unlike any of the 14 Emmer chromosomes and would rarely combine with them. In more fertile combinations of Emmer and Vulgare wheats, such as *T. dicoccum* or *T. turgidum* × *T. vulgare*, some of the 7 additional chromosomes of the Vulgare parent might in some cases pair with the 14 chromosomes of the Emmer parent. The greater compatibility of the chromosomes in such hybrids would not only increase the fertility, but would perhaps result in some relatively fertile segregates combining some of the characters of both parents. With increased sterility, on the other hand, the intermediate types would be rapidly eliminated and the fertile homozygous segregates could be classed as either Emmer or Vulgare types.

The primary 14 chromosomes of the Vulgare wheats are not, in most cases at least, of the same factorial constitution as the 14 chromosomes of the Emmer wheats. This is shown by the various types of Emmer wheats obtained on crossing members of the two groups. For instance crosses of *T. vulgare* × *T. durum* result in some typical *T. dicoccum* segregates and the cross *T. vulgare* × *T. turgidum* results in segregates resembling *T. dicoccum*, *T. durum*, *T. Spelta* and *T. compactum*. *T. polonicum* or *T. turgidum* crossed with *T. vulgare* produces many segregates like *T. durum*, which are homozygous. LOVE and CRAIG (1919) found 2 segregates resembling *T. dicoccoides* in a cross of *T. vulgare* × *T. durum*. Such a result is not unexpected since *T. dicoccoides* has 14 chromosomes. It does not necessarily mean, however, that all 14-chromosome wheats can be obtained in this way.[4] There is also some incompatibility in certain combinations of the 14 bivalents in Emmer × Vulgare crosses as indicated by the partial sterility of certain 14-chromosome segregates.

In view of the fact that morphological and physiological characters of the Vulgare wheats are associated with the 7 additional chromosomes, it is improbable that the desirable characters of the Emmer and Vulgare wheats can be combined in homozygous fertile segregates. This conclusion is substantiated by the results of a large number of breeding experiments in all parts of the world. FARRAR (1898) recognized the value of combining the rust resistance of the Emmer varieties with the bread wheats, and made numerous species crosses with this end in view, but he was apparently unsuccessful in obtaining the desired results. Crosses between the Emmer and Vulgare wheats have also been made by VILMORIN in France, TSCHERMAK in Austria, BIFFIN in England, and by a large number of plant breeders in various parts of the United States. In Minnesota alone (HAYES and GARBER 1921), over 20,000 F_3 segregates of such hybrids have been studied and only a few plants were found which had the morphological characters of the Vulgare parent and were resistant to rust. So far as I have been able to determine no segregate of economic value has been originated by combining the desirable characters of the Emmer group with the desirable characters of the Vulgare group.

[4] I have found 14 chromosomes in *Aegilops ovata*, but Aegilops is almost if not quite sterile with all of the cultivated species of wheat (PERCIVAL 1921). The sterility relationships, taxonomic characters, pollen-grain shape, and pollen-grain size, all support the contention that Aegilops deserves its generic rank.

THE RELATION BETWEEN CHROMOSOME NUMBER AND MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS IN WHEAT SPECIES

The association between chromosome number and morphological and physiological characters found in segregates of *T. vulgare* × *T. durum* is even more striking in the cultivated species of wheat (figure 1). The Einkorn group, consisting of one species, *T. monococcum*, has 7 chromosomes. It is a very homogeneous species with only a few varieties. It is adapted to barren rocky soil where other wheats will not grow. All

1 2 3 4 5 6 7 8

FIGURE 1.—Representatives of the cultivated species of wheat:
Einkorn group: 1. *Triticum monococcum.*
Emmer group: 2. *T. dicoccum;* 3. *T. polonicum;* 4. *T. turgidum;* 5. *T. durum.*
Vulgare group: 6. *T. Spelta;* 7. *T. vulgare;* 8. *T. compactum.*

varieties are bearded, the empty glumes are strongly keeled and the ears are flattened across the face. The Emmer group, consisting of *T. dicoccum*, *T. turgidum*, *T. polonicum* and *T. durum*, has 14 chromosomes. According to PERCIVAL there are about 150 "forms" (agronomic varieties) in this group. These species are in general restricted in adaptability to dry

warm regions and under semi-arid conditions will yield more than other wheats. Most of the varieties in this group are bearded and the beards are usually very long. The empty glumes are strongly keeled, the ears are usually flattened across the face, and the culms are pithy or, if hollow, have thick walls. The Vulgare group, consisting of *T. vulgare*, *T. Spelta* and *T. compactum*, has 21 chromosomes. The species of this group are very adaptable and are grown in all parts of the world where wheat can be grown. PERCIVAL describes more than 500 varieties in this group. Bearded and beardless forms are found in about equal numbers but the beards of the bearded types are relatively short. The empty glumes are slightly keeled, and with the exception of *T. Spelta*, the ears are square in cross section or broader across the face. The culms are usually hollow and thin-walled.

Of even greater importance than the morphological characters is the relation of rust resistance and bread-making qualities in these three groups of wheat species. VAVILOV (1914) classes Einkorn as immune to rust and mildew; species of the Emmer group are classed as resistant, although *T. dicoccum* has both resistant and susceptible forms; and the species of the Vulgare group are classed as susceptible. In 1918 VAVILOV found all Vulgare wheats tested to be susceptible except Black Persian. PERCIVAL'S (1921) results show, however, that Black Persian is a variety of *T. dicoccum*. According to BIFFEN (1907) Einkorn is notoriously rust-resistant and in ERIKSSON'S trials it was the only variety immune to the three common wheat rusts. MELCHERS and PARKER (1922) also find all strains of Emmer and Einkorn to be more or less resistant. The work of these and other investigators (see PERCIVAL 1921) shows that the Einkorn group is very resistant to rust, the species of the Emmer group are relatively resistant, while the members of the Vulgare group, with the exception of *T. Spelta* and a few varieties of *T. vulgare* and *T. compactum*, are susceptible to rust.[5] Similar relations are found in respect to resistance to mildew (*Erysiphe graminis*) and bunt (*Tilletia tritici* and *T. levis*).

Although the *durum* and other wheats in the Emmer group usually have a higher protein content and a greater yield of flour than the Vulgare wheats, they do not possess the quality of gluten necessary for the manufacture of light bread. Some of the *durum* wheats do, however, make a better quality of flour than some of the soft wheats in the Vulgare group.

[5] STAKMAN and LEVINE (1922) find *T. monococcum* relatively susceptible to most of the 38 biologic forms of *P. graminis*. These results, contradictory to a large amount of data collected in Europe and America, may perhaps be attributed to differences in the distribution and prevalence of the various biologic forms of rust. The species of the Emmer group were found to be relatively resistant.

The wheats of the Emmer group are commonly used in the manufacture of macaroni, or mixed with "strong" flour of Vulgare wheats. Most of the Emmer varieties have hard flinty grains, but varieties of *T. turgidum* and *T. dicoccum* have starchy grains and the flour is used in the manufacture of biscuits and pastry. Only in varieties of the Vulgare wheats are the characters found which make a flour of high quality. LECLERC, BAILEY and WESSLING (1918) made milling and baking tests with several species of wheat. Their conclusions are of so much interest that they are quoted.

> "When these flours were baked into bread, the bread made from Einkorn was the least desirable, not only in volume, but in color, elasticity, and in taste or flavor. . . . . That made from Emmer was somewhat better, although not equal to bread made from spring-wheat flour in color, volume and elasticity. The Polish-wheat [Emmer group] bread had a better color than the Emmer bread, but the volume was not quite so large. . . . . The spelt [Vulgare group] bread on the other hand compared favorably in most respects to the ordinary-wheat bread, except that the color was a little darker. There was no difference in taste and appearance from ordinary-wheat bread."

It is evident that the species of wheat with different chromosome numbers are differentiated by distinct morphological characters, by differences in susceptibility to disease, and by differences in quality of grain. As the chromosome number increases, the variability and adaptability of the species increases, its propagation becomes more dependent on artificial methods, its susceptibility to plant diseases increases, and its economic value is greater. A similar correlation is found in the species of oats. VAVILOV (1918) finds *Avena brevis* and *A. strigosa* most resistant to rust in Russia. DURRELL and PARKER (1920) find *A. barbata*, *A. brevis* and a few varieties of *A. sativa* very resistant to crown rust (*P. coronata*). *A. barbata* was also very resistant to stem rust (*P. graminis*). The species *A. brevis*, *A. barbata* and *A. strigosa* have 7 or 14 chromosomes as compared with 21 for *A. sativa* (KIHARA 1919). Here we find, again, greater disease resistance in the species with the smaller chromosome number and greater economic value of species with the increased chromosome number. The relation between chromosome number and the various characters described may be attributed to the greater opportunity for mutations to occur in species with larger chromosome number, to a greater number of possible combinations in fertile hybrids, and to the cumulative effect of reduplicated factors or the combined effect of many different factors (see EAST 1915, and BRIDGES 1922).

THE NATURE OF RUST RESISTANCE

Rust resistance has been found to be dependent on a single hereditary factor in several Vulgare crosses (BIFFEN 1912, ARMSTRONG 1922). The work of ARMSTRONG is especially valuable as his F_2 classification was verified by F_3 data. It is of interest to note that BIFFEN found a simple 3 : 1 ratio for susceptibility to rust in a cross of resistant and susceptible varieties of *T. vulgare*, but that he obtained no Mendelian ratios in a cross of *T. turgidum* × *T. vulgare* in which about 2000 F_2 segregates were obtained. HAYES, PARKER and KURTZWEIL (1920) conclude that linkage prevents free assortment of factors for rust resistance in crosses of Emmer × Vulgare wheats. The non-Mendelian inheritance of rust resistance in partially sterile hybrids can be easily explained in view of the cytological analysis of such hybrids.

It has been suggested that resistance to rust infection may depend on the size of the stomata, the resistant varieties having stomatal openings too small for the hyphae to enter. This view would agree very nicely with the degree of resistance found in the cultivated species of wheat because stomata size is closely associated with chromosome number. Under the same environmental conditions the relative size of stomata for the Einkorn, Emmer, and Vulgare groups is in the ratio of 10, 14 and 18.

Although the assumption that susceptibility is due to the large size of the stomata may be a very attractive hypothesis, the works of WARD (1905), MARRYAT (1907), GARBER (1922) and others show clearly that it is untenable.[6] These investigators find that the rust hyphae enter the stomata of immune species and varieties about as frequently as in the susceptible varieties, or that stomatal size is not associated with resistance. In case the hyphae enter the stomata of such a resistant species as Einkorn the host cells shrink and break down and the hyphae are starved. In less resistant varieties the host cells have a more protracted struggle with the fungus and a few pustules may be formed, while in completely susceptible varieties there is no retardation in the growth and development of the fungus. Susceptibility to rust is apparently dependent directly or indirectly, on physiological factors. The exact nature of these factors is unknown, but VAVILOV (1918) states that immunity to rust is not due to osmotic pressure or hydrogen-ion concentration in the host cells.

[6] Dr. RUTH ALLEN (1923) has recently published a very complete account of the factors involved in rust resistance in wheat. She finds resistance in Kanred wheat to be due to small size of stomatal openings, to heavy walls adjoining pathological cells, and to a true physiological immunity.

The fact that nitrogenous fertilizers, and wide spacing of plants may increase susceptibility (ARMSTRONG 1922) also supports the assumption that susceptibility is due to physiological factors. RAINES (1922) concludes that susceptibility to rust increases as the vegetative vigor of the host increases. Thus, increased vigor in segregates due to heterosis might have considerable effect on susceptibility or resistance. Since so many factors might affect the physiological balance of an individual, especially in species crosses, it is not surprising that unusual results are found and that transgressive segregation frequently occurs in hybrids between susceptible and resistant species or varieties.

The occasional appearance of segregates in Emmer × Vulgare crosses, which resemble common wheat and are also resistant, may be due to physiological conditions, and it is perhaps significant that few if any of these segregates are of economic value. Vegetative vigor, in general, increases as the chromosome number increases, within a given genus, and thus the relative susceptibility of the various species of wheat might be explained. However, genetic studies show that the physiological balance which makes a plant resistant or susceptible may, in the Vulgare wheats at least, depend on a single hereditary factor. ARMSTRONG has found certain varieties immune to rust under all environmental conditions available.

Nearly all varieties of *T. vulgare* and *T. compactum* are susceptible to rust. Marquis was at one time considered resistant, but it was found that it simply escaped rust because of its early maturity. Recently several resistant varieties have been found. WALDRON and CLARK (1919) find Kota to be a resistant variety, but it has from 5 to 15 percent rust infection in North Dakota and is slightly more susceptible in other localities. MELCHERS and PARKER (1922) have made 3 selections of Crimean wheat which are relatively resistant. Kanred, the best of these selections, had from 40 to 70 percent rust in 1915, but usually it is very resistant. Kota and Kanred as well as the other two selections, are very similar in morphological appearance and both belong to the Crimean group of Vulgare wheats which were originally obtained from southern Russia. They are awned, lax-eared, and have comparatively long beaks on the outer glumes. Resistant wheats are not confined to beardless types although these are of most economic value. Fern, a variety of *T. vulgare*, is awnless and is as resistant as Kanred (MELCHERS and PARKER 1922).

A comparison of the resistance of different species and varieties to rust and bunt shows that the factors which cause resistance to rust also cause resistance to bunt. GAINES (1918) finds Turkey, a variety in the Crimean

group, to be relatively resistant to bunt. STEPHENS and WOOLMAN (1922) find most Vulgare wheats susceptible to bunt in Oregon. A large number of Crimean selections from the KANSAS AGRICULTURAL EXPERIMENT STATION were found to be highly resistant. Although most of the resistant varieties were awned Crimean wheats, several awnless varieties were found to be resistant. GAINES (1920) crossed Turkey with Florence, both resistant varieties, and obtained a number of segregates even more resistant than the parents and either awned or awnless resistant types could be obtained.

Through the courtesy of Doctor GAINES data have been obtained for bunt resistance in the various species of wheat. GAINES finds Einkorn to be completely immune. The percentage of infection in *T. polonicum* was 8, in *T. dicoccum* 10, in *T. durum* 29, in *T. turgidum* 33. In the Vulgare group, *T. Spelta* was comparatively resistant with only 10 percent infection, but the percentage of bunt found in *T. vulgare* was 70 and in *T. compactum* it was 64. In general, the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa*. It is probable then, that varieties found to be resistant to rust in the middle west would be valuable disease-resistant varieties for the Pacific coast where bunt is the most important cereal disease. Likewise the valuable results in breeding bunt-resistant wheats on the Pacific coast could be utilized in regions where rust is prevalent. In many cases resistant segregates might not be adapted to the region where they were originated but would perhaps be suited to other climatic conditions.

In selecting disease-resistant varieties of wheat for breeding work the chances for successful results are much greater if the parents are selected within the Vulgare group. Several disease-resistant varieties of *T. vulgare* are known and these can be used in breeding varieties suited to local conditions. *T. Spelta* may also be of value since it is relatively resistant to disease and is very hardy, but it is possible that resistance may be associated with *Spelta* morphological characters even in hybrid segregates.

SUMMARY

Previous investigation has shown that the cultivated species of wheat may be divided into 3 groups according to their taxonomic, sterility and cytological relationships. These groups are the Einkorn group with 7 haploid chromosomes, the Emmer group with 14 chromosomes, and the Vulgare group with 21 chromosomes.

It has also been found that in F_1 hybrids of Emmer × Vulgare wheats there are 14 bivalent and 7 univalent chromosomes in the meiotic divisions. The bivalent chromosomes divide normally in both reduction divisions, but the univalents lag and divide equationally in the first division and pass at random to one pole or the other without dividing in the second division. Thus the F_1 gametes contain from 14 to 21 chromosomes. There is considerable sterility in F_1 and subsequent generations.

A cytological examination of 46 F_3 segregates of *T. vulgare* × *T. durum* shows that most of the segregates have either 14 or 21 chromosomes or an intermediate number which could result from the union of a 14-chromosome gamete with a 21-chromosome gamete. It is evident that the segregates with an intermediate chromosome number tend to be eliminated because of their sterility and that the ultimate homozygous fertile segregates will have either 14 or 21 chromosomes.

A very high degree of association was found between chromosome number and morphological and physiological characters in the F_3 segregates. The plants with 14 chromosomes were similar in morphological characters and rust resistance to the *durum* parent, while segregates with 21 chromosomes had the morphological characters and susceptibility to rust of the *vulgare* parent. Apparently the 7 additional chromosomes of the Vulgare varieties determine the distinguishing characters of the common wheats due to a reduplication of hereditary factors or to specific factors in these chromosomes.

The cytological and genetic data would indicate that many of the desirable characteristic properties of the Emmer and Vulgare wheats cannot be combined in a homozygous condition. This conclusion is supported by breeding experiments in all parts of the world and involving hundreds of thousands of segregates. So far as the writer has been able to determine no variety of economic importance has been originated by combining the desirable characters of the Emmer and Vulgare wheats.

There is a striking correlation between chromosome number and morphological and physiological characters in the cultivated species of wheat. With an increase in chromosome number, 7–14–21, there is an increase in variability and adaptability, an increased susceptibility to rust, mildew and bunt, a better quality of gluten in the grain, and the economic value is greater. The relation between chromosome number and morphological and physiological characters may be attributed to the greater opportunity for mutations to occur in species with the larger chromosome number, to a greater number of possible combinations in

fertile hybrids, and to the cumulative effect of reduplicated factors or the combined effect of many different factors.

The resistance to rust appears to depend on the physiological condition of the host. This condition may depend on a single hereditary factor in some varietal crosses, but in species hybrids many factors, acting either directly or indirectly, may influence the physiological balance which determines resistance or susceptibility. Rust and bunt resistance apparently depend on the same factors, so that results in breeding wheats resistant to rust can be applied to bunt, and *vice versa*.

Pollen-grain size may be used as an approximate measure of chromosome number in wheat species and hybrid segregates.

The breeding of wheat varieties to combine disease resistance with high yield and quality of grain is much more likely to be successful if the parents are selected within the Vulgare group.

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ON "MATERNAL INHERITANCE"

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INTRODUCTION

There is a type of heredity known as "maternal inheritance" because the characters are influenced more by the egg than by the spermatozoön; but they are proved, nevertheless, to have their own allelomorphs and to be subject to Mendel's law of segregation.

Since the late Prof. TOYAMA (1913) used the term "maternal inheritance" for the first time in his study on the egg color of the silk-worm, *Bombyx mori* L., much interest has been centered on the problem and it came to be believed by some that there is really such a special type of heredity. But it seems that the study has been limited chiefly to the egg color (TOYAMA 1912, 1913, TANAKA 1919) and voltinism (TOYAMA, 1912, 1913, MCCRACKEN 1909, CASTLE 1910, CLEGHORN 1918, WATANABE 1918, 1919) in the silk-worm. Besides these, there are some characters which have been erroneously treated as maternal inheritance. Indeed, cases of maternal inheritance are apt to be confused, not only with plastid inheritance,[1] but also with the phenomena of BATESON's "mono-

[1] If there are self-perpetuating materials in the cytoplasm, they must be inherited chiefly through the egg cell; and, in fact, there is excellent genetic evidence that a peculiar kind of inheritance in certain chlorophyll characters is due to the transmission of self-perpetuating bodies called plastids in the cytoplasm. Therefore I recognize the plastid inheritance as one type of heredity, and cannot agree with EMERSON (1914) who ascribes its cause to the variability of unit factors, nor with PLATE (1913) who calls it "falsche Vererbung."

lepsis," or with cases of inheritance in which all or some of the paternal chromosomes fail to function, leaving the embryo with its maternal set alone.

Consequently, all the definitions of maternal inheritance are very ambiguous and no one has established clearly its *raison d'être* as a type of heredity. Recently MORGAN (1919) has given a definition as follows:

" 'True' maternal inheritance relates to peculiarities of the egg or larva that are due to materials already present in the egg-cytoplasm when the egg is laid."

I cannot agree with him when he establishes, without tracing its causes, a special type of heredity based upon a peculiar phenomenon concerning only the characters of the egg or larva,—that is, the characters of earlier stages of development. TANAKA (1919) studied the inheritance of the egg color of the silk-worm and stated the characteristics of maternal inheritance as follows:

"Segregation is found among the batches,—that is, there are two kinds of mother moths, one of which lays only the dominant-colored eggs and the other lays only the recessive-colored ones. In other words, dominant- and recessive-colored eggs do not mix in a batch."

But the author of the present paper has been able by the following experiments to show this proposition to be false.

Now the most typical instance of maternal inheritance heretofore known is the brown or reddish-brown color of the silk-worm eggs which was first studied by TOYAMA. But his study consisted chiefly in the selection of the brown-colored race and was lacking in the detailed crossing experiments. TANAKA (1919), basing his conclusions on TOYAMA'S study of his crossing experiments, came to the wrong conclusion, as quoted above.

I could not help doubting the existence of maternal inheritance as a type of heredity; and to clear up this question I have studied, since 1918, crossing experiments on the brown-colored eggs of the silk-worm. I have been able to show that, if we suppose a time is needed for every unit factor to manifest its own action, i.e., "time of action," we can clearly understand the so-called maternal inheritance without any peculiar assumption. I am firmly convinced that it is not irrational to suppose that "time of action" is required for everything, and that such assumption does not conflict with physical nor chemical principles. The following series of experiments will throw some light upon this matter.

Before giving details of my investigations, I wish to express my heartfelt thanks to Prof. Dr. CHIYOMATSU ISHIKAWA, Prof. Dr. E. G. CONKLIN

and Prof. Dr. G. H. SHULL, for their valuable advice and their kindness in revising the manuscript.

MATERIALS, METHODS AND REMARKS

The brown-colored race here used was first selected by TOYAMA from a Japanese divoltine white race and since 1916 has bred true for 12 generations to its brown-colored character of the eggs laid.

As the material of the normal race, which has slate-colored eggs, I adopted a Japanese divoltine white race called "Yamatonishiki" which bred true to its normal-colored character of the eggs for 16 generations.

As TOYAMA mentioned, the color of the shell and yolk of both variants are the same and we must attribute the chief cause of those characteristics to the pigments in the serosa, a product of the combination of both parental gametes.

The artificial method of hatching and method of breeding I adopted are quite the same as those mentioned in my previous paper (UDA 1919), so there will be no need of repeating them. But I must mention here the reason why I have adopted the divoltine races as material for the investigation, in spite of the disadvantage that the color of their non-hibernating eggs is yellowish white, since no pigments are produced in the serosa, and it is thus impossible to detect the zygotic constitution with respect to the egg color. Notwithstanding this difficulty, the divoltine races are satisfactory for such studies, because, as has recently been made known from WATANABE'S (1918, 1919) experiments, even the divoltine races may be made univoltine by raising the incubating temperature to 30° C. It is quite advantageous therefore to use them for the experiment, since we can on the one hand study the egg color in each generation, and on the other hand, we can raise two generations in a year without any artificial method of hatching.

For the sake of convenience I use the following abbreviations:

"Brown" refers to brown-colored eggs, and in some cases also to the moth which lays brown-colored eggs.

"Normal" refers to normal or slate-colored eggs, and in some cases also to the moth which lays normal-colored eggs.

"Mixed" refers to the mixed batch of brown and normal eggs.

CROSSES BETWEEN BROWN ♀ AND NORMAL ♂

F_1 generation

All were brown throughout all the batches, and I was unable to detect any difference between the pure brown parent and F_1 hybrids, in the intensity of the brown color of the eggs.

F_2 generation

The F_2 generation produced by the F_1 hybrids above described were found to contain normal and brown in the ratio shown in line 1 of table 1; but these brown are a little different from those of the F_1 hybrids, i.e., they are darker than the pure brown. In other words, they apparently

TABLE 1

Summaries of 28 breeding experiments involving normal (i.e. slate-colored) and brown egg color in silk-worms.

NUMBER OF TABLE	NUMBER OF HATCHES	NORMAL	BROWN	TOTALS	RATIOS	DEVIATION	PROBABLE ERROR (P.E.)	DEVIATION / P.E.Dev.
1	166	73,358	24,431	97,789	3.0007:0.9993	±0.0007	±0.0037	0.19
2	63	24,963	8,554	33,517	2.9791:1.0209	±0.0209	±0.0064	3.27
3	11	3,969	1,354	5,323	2.9825:1.0175	±0.0175	±0.0160	1.09
4	22	6,845	6,734	13,579	2.0163:1.9837	±0.0163	±0.0116	1.41
5	267	114,070	38,409	152,479	2.9924:1.0076	±0.0076	±0.0030	2.53
6	43	14,828	5,111	19,939	2.9747:1.0253	±0.0253	±0.0083	3.05
7	3	757	249	1,006	3.0100:0.9900	±0.0100	±0.0368	0.27
8	9	3,593	1,194	4,787	3.0023:0.9977	±0.0023	±0.0169	0.14
9	24	6,610	6,853	13,463	1.9639:2.0361	±0.0361	±0.0116	3.11
10	24	8,950	3,090	12,040	2.9734:1.0266	±0.0266	±0.0106	2.51
11	23	5,658	5,633	11,291	2.0044:1.9956	±0.0044	±0.0127	0.35
12	11	4,766	1,614	6,380	2.9881:1.0119	±0.0119	±0.0146	0.82
13	12	3,328	3,376	6,704	1.9857:2.0143	±0.0143	±0.0165	0.87
14	2	556	531	1,087	2.0460:1.9540	±0.0460	±0.0409	1.12
15	73	18,362	18,908	37,270	1.9707:2.0293	±0.0293	±0.0070	4.19
16	4	1,602	553	2,155	2.9735:1.0265	±0.0265	±0.0252	1.05
17	16	4,329	4,551	8,881	1.9498:2.0502	±0.0502	±0.0143	3.51
18	96	37,915	12,881	50,796	2.9857:1.0143	±0.0143	±0.0052	2.75
19	36	13,155	4,449	17,604	2.9891:1.0109	±0.0109	±0.0088	1.24
20	57	24,642	8,222	32,864	2.9993:1.0007	±0.0007	±0.0064	0.11
21	7	1,829	1,813	3,642	2.0088:1.9912	±0.0088	±0.0224	0.39
22	21	8,468	2,927	11,395	2.9725:1.0275	±0.0275	±0.0109	2.52
23	19	5,202	5,351	10,553	1.9718:2.0282	±0.0282	±0.0131	2.15
24	4	1,420	488	1,908	2.9769:1.0231	±0.0231	±0.0267	0.87
25	5	1,388	1,427	2,816	1.9723:2.0277	±0.0277	±0.0254	1.09
26	3	943	320	1,263	2.9865:1.0135	±0.0135	±0.0329	0.41
27	6	1,999	682	2,681	2.9825:1.0175	±0.0175	±0.0226	0.77
28	2	562	509	1,071	2.0990:1.9010	±0.0990	±0.0412	2.40

assume an intermediate color between slate and brown. On the contrary, we cannot detect any difference between the pure normal and the F_2 hybrids in the intensity of the normal color of their eggs.

Thus, contrary to TANAKA (1919), it will be seen that in F_2 there are found both normal and brown in all the batches exactly in the ratio

3 : 1. Consequently, it is shown to be a mistake that in F_2 we have only the dominant type,—which has hitherto been regarded as the characteristic of maternal inheritance.

It is seen throughout all of my experiments that one moth lays only normal eggs, another lays only brown eggs, a third lays normal and brown eggs mixed. The normal eggs of the first and the third cases are quite the same as the pure normal, and the brown eggs of the second case are quite the same as the pure brown. But the brown eggs of the third case are always darker than the pure brown and appear intermediate between the normal and the brown as above mentioned. For the sake of convenience I have named them brown in the tables.

F_3 generation

It was found that brown in the previous generation (F_2) produced brown offspring without a single exception.

Some of the normal in F_2 produced exclusively normal offspring and the others were found to segregate in the ratio, 3 normal : 1 brown, as summarized in line 2 of table 1.

F_4 generation

It was found that brown in the previous generation (F_3),—even those in the mixed batches,—produced brown offspring without a single exception.

Normal from pure-bred in the previous generation produced only normal offspring, i.e., they bred true to normal. But normal from the mixed batches were found to behave as those of F_3; namely, some of them produced exclusively normal offspring while the others segregated in the ratio 3 normal : 1 brown. (See line 3 of table 1.)

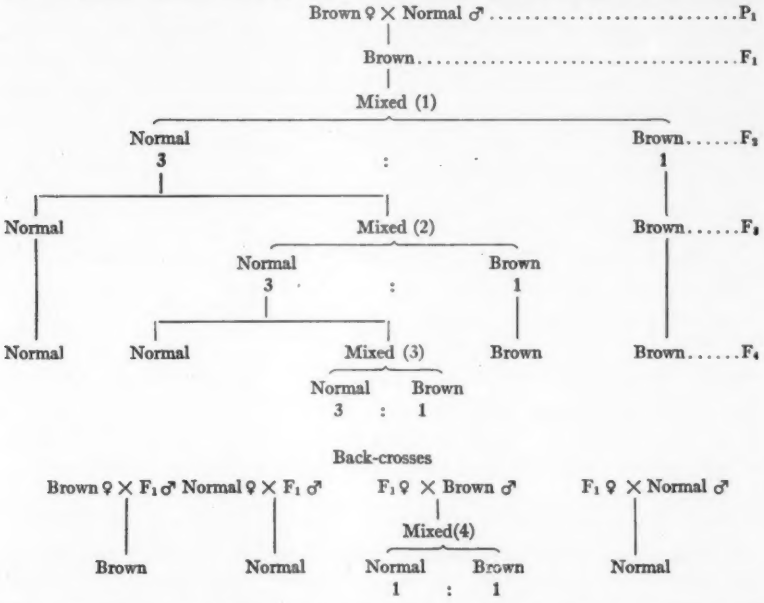
Back-crosses

I back-crossed the cross-bred form with both of the pure parent breeds, and found $F_1 \times$ normal and its reciprocal produced normal only. In the case of $F_1 \times$ brown a segregation occurred in the ratio 1 normal : 1 brown as line 4 of table 1 shows, but its reciprocal, i.e., brown ♀ $\times$ F_1 ♂, produced exclusively brown offspring.

The above-mentioned series of experiments are shown briefly and clearly in the diagram on the opposite page. The Arabic numeral in parenthesis at the right of the "Mixed" in these diagrams denotes the number of table which shows the segregating ratio in that mixed batch. To save space and expense the tables of original data have been merely summarized in table 1 accompanying this paper.

CROSSES BETWEEN NORMAL ♀ AND BROWN ♂

The reciprocal crosses yielded results practically the same as those above-mentioned, excepting that in the F_1 generation I obtained exclusively normal-colored eggs in all batches, and that some of the normal



from pure-bred in F_2 segregated in the ratio 3 normal : 1 brown; the whole of which results are better shown briefly in tabular form on page 328.

DISCUSSION AND CONCLUSIONS

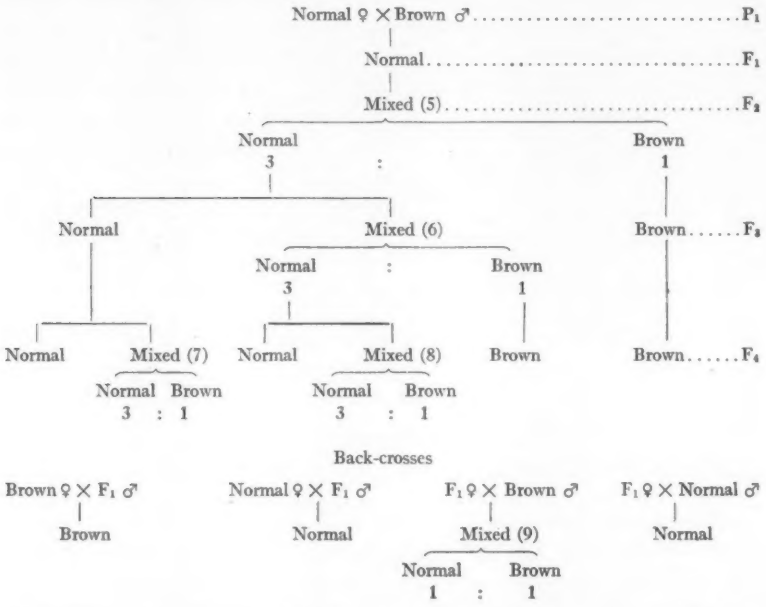
We can consider from the series of experiments above-mentioned that normal and brown are allelomorphic with each other, the former being dominant over the latter. But the following two points are peculiar to these experiments and conflict with the Mendelian expectation:

(1) In the F_1 generation the eggs manifest in color entirely the maternal character, whether it was dominant or recessive.

(2) The back-cross of brown ♀ × F_1 ♂ produced brown only, and did not segregate in the usual ratio, i.e., 1 : 1.

In order to make this point clearer I investigated the color of eggs produced by crossing F_1, F_2, F_3 and back-cross, in succession, with the

pure recessive breed, brown. Besides these, I studied the next generation of the back-crosses which revealed their zygotic constitution. These results will be clear from the diagram on page 330. The arrow indicates the direction of the cross, in each case pointing from the male to the female of the given cross.



The general hereditary phenomena here which apparently conflict with Mendelism may be summarized as follows:

(1) When we use a female of one of the pure breeds, brown or normal, as the mother moth, the eggs laid are always the same in color as those of the pure breed to which the mother belongs, no matter whether the egg characters of the paternal breed be dominant or recessive, and also whether his zygotic constitution with respect to egg color be homozygous or heterozygous. In other words, the condition of the blood with reference to the character in question is due to the condition of the mother regardless of the condition of her mate.

(2) Even from crosses between moths developed only from the brown —the recessive—eggs, we sometimes obtain the mixed batches. And the ratio of normal to brown is 3 : 1 or 1 : 1.

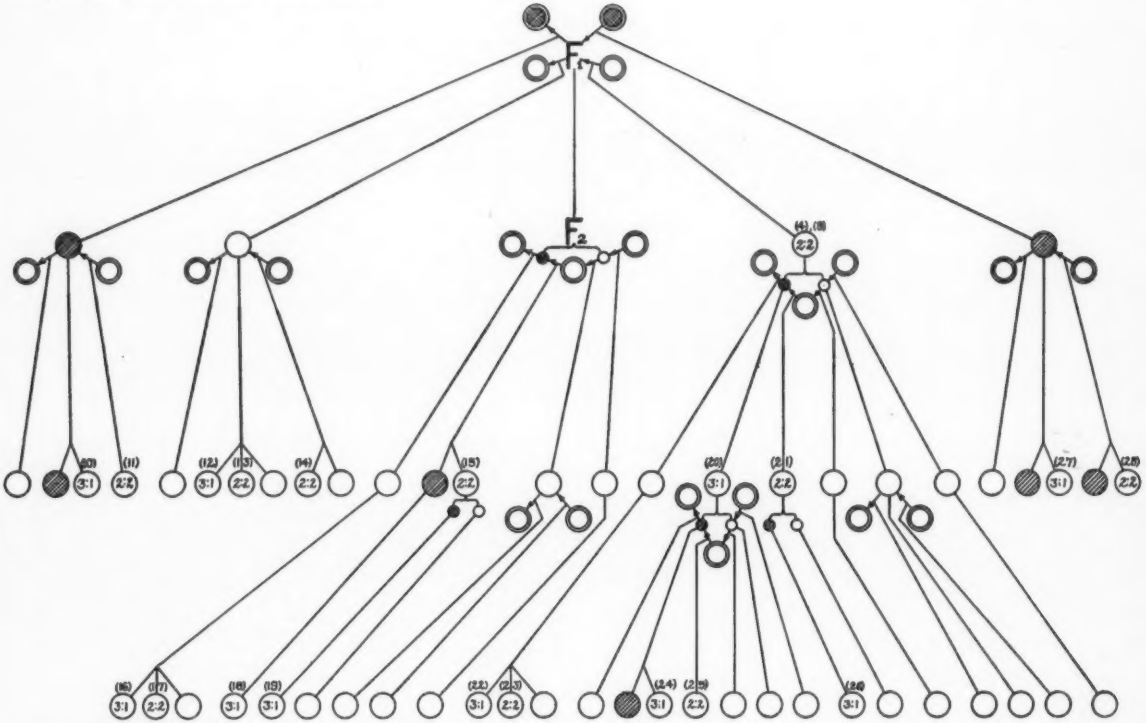
These conditions cannot, indeed, be found in the common types of inheritance, but it would be hasty to conclude immediately from these facts that a new type of heredity is involved. The segregating ratio of 3 : 1 in F_2, F_3 and F_4, and the ratio of 1 : 1 in the back-crosses can be explained only by Mendel's law of segregation, assuming that normal and brown are allelomorphic to each other. And the fact that the brown eggs in the mixed batches were found, by crossing *inter se*, to breed true to their type, is also an indication that brown is a recessive allelomorph, in accord with Mendelism. Then, what leads to the peculiarities noted above? The following in my opinion will solve the riddle of such peculiarities and will perhaps be acknowledged to be a general phenomenon of heredity.

It will be clear from the above experiments that, while the eggs homozygous for the normal factor always give rise to slate-colored eggs, the eggs heterozygous for the same factor sometimes manifest the recessive color, brown. Such an anomalous heterozygous type is limited to the cross-bred form between the pure brown ♀ and the pure normal ♂, the reciprocals, on the contrary, being always of normal color. But, as both of these crosses are quite the same in the segregating phenomena in the subsequent generations and in the back-crosses, *the zygotic constitution is clearly the same in both.* In other words, while the eggs differ in color, *the moths developed from them lay quite the same-colored eggs.*

Next, while the F_1 brown eggs are similar in color to those of the pure brown, the F_1 female moth arising from the brown eggs gives, when fertilized by a pure normal male, the ratio of 3 normal-colored to 1 brown egg; the female from the pure brown eggs, on the contrary, gives only brown eggs with the male moth of the pure normal. Consequently we can see that the pure brown and the heterozygous brown are similar to each other in the phenotypic color of eggs from which they develop, but differ from each other genotypically, as shown by the color of the eggs they lay.

Thus, we know that the character of the moth, with respect to the color of the eggs it lays, coincides with the expectation from its zygotic constitution and thus falls in perfectly with Mendelism, but it is not always so with the color of eggs from which the moth developed. In other words, the factors for the brown and the normal manifest their own effects only in the later stages of development. And the cause of the anomaly in the early stages of development, i.e., in the egg stage, is owing to the fact that the factor for the egg color in the male gamete, being

UDA, HAJIME, "ON MATERNAL INHERITANCE"



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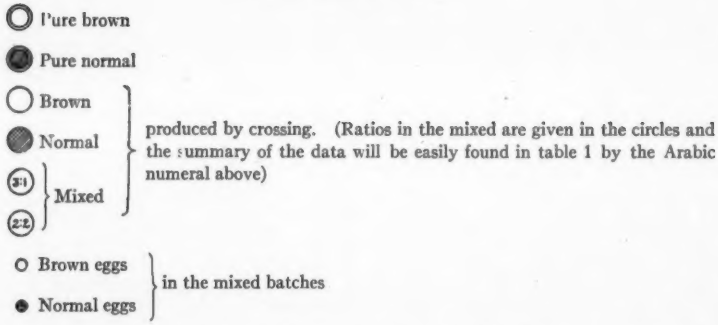
LEGENDS FOR PLATE 1


Pure brown
Pure normal
Brown
Normal
3:1
2:2
Mixed
produced by crossing. (Ratios in the mixed are given in the circles and the summary of the data will be easily found in table 1 by the Arabic numeral above)
Brown eggs
Normal eggs
in the mixed batches

The arrows indicate the direction of the crosses, in each case pointing from the male to the female of the given cross.

introduced into the egg at the moment of the fertilization, has not time enough to manifest its own effect even if it be dominant.

In almost all the hereditary phenomena hitherto known, both in animals and plants, the characters which are repeated generation after generation according to the Mendelian formula relate to the later stages of development. And the cases which appear to show no male effect, relate to the characters in the early stages of development, i.e., chiefly the characters of the egg or the larva in animals and those of the seed or the cotyledon in plants.

Such being the case, I am firmly convinced that we can generalize the above interpretation; that is, a certain time is generally required for every unit factor to manifest its own action, i.e., "the time of action."

Although such an assumption has never been clearly stated, it has been sometimes vaguely considered by a number of previous authors.

DRIESCH (1903), BOVERI (1903) and CONKLIN (1915) have found that in the crosses of the sea urchins the male effect is first noticed when the larval skeleton is formed. In the crossing experiments with White and Black Leghorns, DAVENPORT (1906) states that white becomes perfectly dominant over black in the grown-up fowls, while the young heterozygous chicks show some dark pigment. LYNCH'S (1919) study has clearly shown that the effect of a lethal factor in Drosophila is manifested after the larval stages.

MORGAN (1914) used the term "deferred nuclear influence," explaining the results of CONKLIN'S experiments on Cynthia and those of Miss PINNEY'S (1918) observations on the many crosses between different species of fishes, both of which are cases where no paternal influence is manifested during the early embryonal stages. Would it not be more reasonable in these cases to think that these are rather the natural results of a certain "time of action" required for the manifestation of the paternal factors than to vaguely ascribe them as the results of the "delay" of the nuclear influence?

Now, the results of all our experiments will be clearly accounted for upon such an assumption; and the two points mentioned which apparently conflict with Mendelism will also be cleared up, for it will be at the later stage of development, i.e., the imago stage,[2] that the factors for normal and brown will manifest their effects upon the egg cytoplasm.

[2] The full development of the capacity to produce brown or slate-colored eggs may be attained at some other stage between the egg and the imago, but there is no way to determine this exactly, because the factors in question both relate only to the color of the eggs.

Furthermore, the fact that the brown eggs in the mixed batches are darker than the pure brown and apparently assume an intermediate color between normal and brown may be easily understood if we consider that the cytoplasm of the egg was already affected by the action of the egg nucleus of the normal.

On the whole, we are, I believe, justified in denying the existence of "maternal inheritance" as a special type of heredity. And I cannot help admiring the keen observation of BATESON (1913, p. 264) who stated that:

"Seeds are in botany what larvae are in zoölogy, and no example is yet known in which the maternal impress extends beyond the seed stage."

Next we shall briefly touch on the hereditary phenomena of voltinism in the silk-worm, which has hitherto been known as the other striking case of so-called maternal inheritance.

As known for a long time the voltinism is very easily changed by the environment, especially by the incubating temperature. My extensive observations prove that there are often found two types differing in their voltinism in a single batch. So it is very doubtful, I think, that differing voltinism may be due to different allelomorphic factors and that it is transmitted according to maternal inheritance. But, even if it be a fact that each type of voltinism has its own factor, the crossing experiments hitherto made on voltinism may be easily understood by the assumption of "time of action." And in this case we can also see that the "time of action" may be varied by the environment. This explanation will surely be more defensible than the establishment of a peculiar type of inheritance.

In connection with my hypothesis, I shall add a few words in conclusion on the theory LOEB (1916, 1917) has recently published, which may be summarized as follows (LOEB 1916, p. iv):

"The egg (or rather its cytoplasm) is the future embryo upon which the Mendelian factors in the chromosomes can impress only individual characteristics."

Though such an idea was also maintained by BOVERI (1903) and CONKLIN (1915), it must be admitted that the cytoplasm is in many cases not able to manifest its action without the presence of a nucleus. A number of facts which show the action of the nucleus in regeneration, assimilation and in many other physiological phenomena of cells, clearly reveal the importance of the nucleus. Is it not then more reasonable to think that the cytoplasmic differentiation observed in many eggs before the entrance of a spermatozoön is, after all, produced by the action of the egg-nucleus?

Can we not also consider that the phylogenetic stages characteristic of the phylum, class, order, family, genus and species, which appear at the earlier stages of development, are determined by the egg-nucleus, while the characters which become manifested at the later stages are the result of the action of both the paternal and maternal nuclei?

Thus I think that the essential substance which brings about differentiation of the cells in development, either at the earlier or later stages, is derived only from the nucleus, and manifests itself in the phenotype after a suitable "time of action."

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PHYSIOLOGICAL POLARITY AND SYMMETRY IN RELATION TO HEREDITY

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INTRODUCTION

The problem which at present we formulate in terms of heredity *versus* environment has, in one form or another occupied the attention of biologists since the time of ARISTOTLE. In earlier times commonly the question was whether the organism is wholly preformed, but it has undergone various changes as regards its terms and the meanings assigned to them. Nearly thirty years ago WHITMAN (1895) pointed out that it was no longer a question of preformation *versus* epigenesis, but rather one of the part played by each factor in determining the individual. Even then the chief exponent of preformation, WEISMANN, was forced to admit the effect of environment, as is evident at various points in his writings, and OSCAR HERTWIG, perhaps his most notable opponent, postulates as necessary for the development of the different species of organisms, "different sorts of primordial substances which possess an extremely complex organization" and which, because of this organization are capable of reacting specifically and with the greatest exactness to all external and internal stimuli to which they are subjected (HERTWIG 1894, p. 131). With the development of experimental biology during the last thirty years, the chief difficulty of preformistic theory has been to account for the variations and modifications in individual development with change in environment, while epigenetic theory has found it difficult to account for the constancy of development and individual pattern, but on the whole there has been still further approach to a common ground.

In the minds of the earlier preformists there was no doubt that the course of individual development was predetermined. In more recent times ROUX advanced the hypothesis of qualitative nuclear division as a basis for the orderly and harmonious differentiation of parts and WEISMANN adopted this idea. Later, however, the experimental investigations of DRIESCH and many others led ROUX himself to abandon the idea, and even the more extreme preformistic school of the present day maintains on the basis of chromosome behavior in cell division, as well as of experimental embryology, that no such sorting out or distribution of genes, factors, hereditary potentialities, occurs. Each cell is regarded as possessing the entire chromosomal mechanism and therefore, as MORGAN puts it, "each cell *inherits* the whole germ plasm" (MORGAN 1919, p. 241). In short, current preformistic theory provides no mechanism for individual development and differentiation. MORGAN'S discussion of "the organism as a whole, or the collective action of the genes" (MORGAN 1919, pp. 241–246) does not provide us with any theory of development. The chromosome theory of heredity tells us that each cell inherits the whole germ plasm, but as to the manner in which different cells and cell groups become different, it has nothing to say.

In an earlier publication MORGAN and his co-workers put the case as follows (MORGAN, STURTEVANT, MULLER and BRIDGES, 1915, p. 43).

> "We must suppose then, that the Mendelian factors are not sorted out, each to its appropriate cell, ... but that differentiation is due to the cumulative effect of regional differences in the egg and embryo, reacting with a complex factorial background that is the same in every cell."

Presumably cytoplasmic differences are meant here, and in his later book MORGAN says that "the evidence from embryology appears to show that the reactions by means of which the embryo develops, and many physiological processes themselves reside at the time in the cytoplasm" (MORGAN 1919, p. 219). But though he admits the possibility of cytoplasmic, as well as chromosomal inheritance, he does not offer any theory of development, even on a cytoplasmic basis, and to judge from his identification of the "organism as a whole" with the "collective action of the genes" (see above), he is inclined to regard the cytoplasmic differences as originating in some way through the action of the genes.

THE PROBLEM OF INDIVIDUAL PATTERN AND DEVELOPMENT

The theory of development advanced by WEISMANN has been discarded and it is evident from what has been said above that more recent theories of heredity offer nothing in its place. If then we accept these

theories of heredity, we must provide a theory of development quite apart from them. That is to say that preformistic theory has at last reached the position where it concerns itself only with the pattern of the hereditary potentialities, genes, factors, or whatever we prefer to call them, and not with the pattern of the individual organism. This being the case, the question at once arises whether the pattern of the individual is in some way predetermined in the pattern of the hereditary potentialities or whether some other factor is concerned in the orderly and harmonious realization of potentialities in the individual. As regards what we may call the specific features of individual pattern, there seems to be no doubt that they are in some way and in some degree predetermined in the hereditary pattern. Such specific features are, for example, those in which Paramecium differs from a planarian, a hydroid from a sea urchin, a crayfish from a bird, or in more closely related forms, the species of a genus or the varieties, races or lines of a species from each other. We believe that the protoplasm, the "germ plasm" of each species and perhaps that of each individual, has a more or less specific physico-chemical constitution which is essentially hereditary, though probably subject in some degree to alteration by action of environmental factors.

There are, however, certain other features of individual pattern which are non-specific in the sense that they are essentially similar in many different species and in certain respects even in plants and animals. These non-specific features of individual pattern are commonly known as physiological polarity and symmetry. They are concerned in the spatial localization and order of both structure and function in the individual. If "each cell inherits the whole germ plasm," as current theory maintains, it is evident that polarity and symmetry are factors of fundamental importance in determining the realization of different hereditary potentialities, the activation of different genes or hereditary factors, in different cells or cell groups. In short, polarity and symmetry apparently constitute the physiological basis of individual, as distinguished from germ plasm pattern: they are concerned in some way in the process by which different cells or cell groups, even though alike as regards constitution of germ plasm, become different in definite orderly ways in individual development. With the appearance of the orderly differences, whether between regions of the cytoplasm of a single cell or between different cells or cell groups, orderly physiological correlation, i.e., the action of one region, cell or cell group upon another or others becomes possible and "the organism as a whole" exists. Physiological correlation may be accomplished either by the formation and transport of substances in mass or by

the transmission of energy changes, but it is evident that such correlation is impossible until differences of some sort exist between the parts concerned.

There is of course nothing new in the idea that polarity and symmetry do not fall into the same category as the hereditary potentialities commonly regarded as constituting the germ plasm; in one form or another the idea has been expressed repeatedly and by many different authors. The conception of development as a mosaic and the hypotheses of formative substances and their localization or stratification involve the idea, and the names of ROUX, E. B. WILSON, MORGAN, CONKLIN, F. R. LILLIE, may be mentioned in this connection, though others might well be added. CONKLIN, for example, has repeatedly emphasized the fact that polarity and symmetry appear in the cytoplasm, though he seems to regard them as at least in part inherent in the cell and perhaps as originating in the nucleus, since he suggests that the localization of substances in the cytoplasm arises largely through the interaction of nucleus and cytoplasm (CONKLIN 1908, 1910, 1914, 1917a, b, 1920, pp. 388-403, 1922, pp. 199-209) although in some of his work he apparently admits the possible action of environment. F. R. LILLIE (1908) showed, however, that polarity and symmetry are functions of the ground substance of the cytoplasm and independent of the localization of the visible substances.

All organisms are orderly as regards the realization of hereditary potentialities in the organization of the individual, and polarity and symmetry, so-called, appear to be the most general and fundamental conditions of such orderly character. It is of importance, however, for any conception of polarity and symmetry to note that in their most general manifestations as polarity and symmetry of whole organisms, they do not seem to be very closely associated with the specific differences of different protoplasms. All except perhaps the simplest organisms exhibit polarity in their pattern, and biologists in general believe that polarity is essentially the same in nature in all the different protoplasms. In fact, many have maintained that polarity is a universal inherent property of living protoplasm. As regards symmetry also we find essentially similar patterns in very different protoplasms, e.g., radial arrangements of branches and other parts in plants and hydroids, bilaterality in flatworms and liverworts, etc. In fact, so far as we know at present there are in all living organisms only three fundamental types of spatial pattern or order: the surface-interior pattern (CHILD 1921 a, pp. 60–64), which is geometrically an order with reference to a point and physiologically an order with reference to an exposed surface; the axiate or polar pattern, geometrically referable to a

line, physiologically referable to a differential of some sort in a certain direction; the bilateral pattern, geometrically referable to a plane, physiologically referable to a ventro-dorsal or dorsiventral differential. These three types of spatial order appear in various combinations and modifications in different organisms. The surface-interior pattern alone gives spherical symmetry and this seems to be the pattern of many of the microorganisms and perhaps also of many cells. The combination of axiate and surface-interior pattern gives what we call radially symmetrical organisms and the combination of surface-interior, axiate and bilateral pattern, bilateral forms. Spiral and asymmetric modifications of pattern also occur, but these do not alter the important fact that the fundamental types of spatial order are only three.

It is extremely improbable that spatial patterns determined by the specific constitution of different protoplasms could be limited to these three simple types. We should expect much greater diversity of pattern if it were determined in that way. The work of REICHERT and BROWN (1909) on the crystallography of the hemoglobins is highly suggestive in this connection as showing the diversity of form in a pattern which is somewhat closely associated with specific physico-chemical constitution. If physiological polarity and symmetry were as closely associated with the specific constitutions of different protoplasms as the crystal forms of the hemoglobins are with their specific constitutions, we should expect a much wider range of diversity in general axial relations in different organisms than the three types of pattern which we actually find.

It seems evident at any rate that polarity and symmetry, the physiological basis of the spatial pattern of organisms, do not fall into the same category as other hereditary factors in the germ plasm. Each cell supposedly inherits the whole germ plasm, but polarity and symmetry represent the pattern of differences between different regions, cells, or cell groups, at least as regards their cytoplasm. On the other hand, parts which have undergone a certain sort of differentiation in relation to polarity and symmetry, may, when isolated, lose this differentiation more or less completely and reconstitute whole individuals. In such cases polarity and symmetry may either persist from the parent individual or be determined *de novo* by certain sorts of relation to environmental factors.

To sum up: physiological polarity and symmetry are the most general and fundamental features of spatial organismic pattern and appear not to be closely associated with the specific constitution of different protoplasms; according to current conceptions of the germ plasm they cannot be repre-

sented solely by genes or factors in it, for "each cell inherits the whole germ plasm" while polarity and symmetry are concerned in some way with the differences between different regions, cells and cell groups; and finally, in development under the usual conditions the axial relations are constant to a high degree, but in many forms they are readily altered by various changes in environmental conditions.

ARE POLARITY AND SYMMETRY INHERENT PROPERTIES OR PHYSIOLOGICAL REACTIONS OF SPECIFIC PROTOPLASMS?

As regards the origin of polarity and symmetry, it is evident that they must either be inherent, predetermined and hereditary, or in some way an effect of external factors. Without going into historical detail, it may be noted that the belief in the inherent, hereditary character of polarity and symmetry has been wide-spread among biologists in the past. These predeterministic conceptions have usually assumed a molecular or "micellar" structure and orientation, either similar or analogous to that of the crystal or that of the magnet, or of purely hypothetical character, as the basis of polarity and symmetry. Such theories of organismic form have been the subject of much discussion and the analogies between crystals and organisms have been stated repeatedly.[1] But even though the crystal does possess a characteristic form and is able to grow, regenerate and undergo "form regulation," the hypothesis that polarity and symmetry and organismic form are fundamentally similar to the spatial pattern of the crystal meets with many difficulties. Some of these difficulties are briefly as follows: according to stereochemical theory, we should expect organisms to show at least as great a diversity of fundamental axial relations as we find of crystal forms and we should also expect these axial relations to be among the most stable and least readily modifiable characteristics of the species. Actually, however, we find, as already noted, only three fundamental patterns and it is well known that these are not only readily modifiable, but modifiable by factors which we cannot readily conceive as having any effect upon molecular or micellar structure and orientation. Again, the crystal is fundamentally a homogeneous system and the occurrence of chemical change in it is accompanied by the disappearance of crystalline structure. In protoplasm, on the other hand, an extreme degree of heterogeneity exists and growth, maintenance, structure, differentiation and function are all associated with, and dependent

[1] See, for example, PRZIBRAM 1906, 1921, for bibliography and for arguments in support of the essential similarity between crystalline and organismic form. The latter paper is concerned to a considerable extent with a hypothetical space lattice.

upon, chemical reactions. Is not the assumption of an inherent molecular or micellar structure and orientation as the basis of organismic pattern in such a system *a priori* a highly improbable one? Such pattern is built up by the metabolic reactions; by altering metabolic relations in different regions or cells we can alter it; when the reactions cease only the formal, not the functional, pattern remains. Moreover, experimental data cited below show that, except with the aid of unwarranted and highly improbable assumptions, it is impossible to interpret the behavior of isolated pieces of organisms in terms of such a molecular or micellar system.

If, however, such a system does exist as the basis of polarity and symmetry and so of organismic pattern, it ought to be possible to obtain some evidence of its existence with the aid of polarized light. Unless the structure of such systems is the same in all directions, they must possess optical axes and under proper conditions show some degree of optical anisotropy, and if such systems are the basis of polarity and symmetry, it is certain that their structure must be different in different directions in most protoplasms. During the nineteenth century extensive studies of the most various animal and plant tissues and structures with the aid of polarized light were made by many investigators,[2] and it was demonstrated beyond question that many animal and plant structures are optically anisotropic. But such structures are predominantly cuticular, non-protoplasmic membranes, shells, skeletal structures, starch grains, crystalloids and fibrillar differentiations, such as muscle, connective tissue, etc., and the investigators agree that protoplasm in general, eggs and early developmental stages, show no indications of anisotropy. The structure underlying anisotropy evidently arises secondarily in the course of development, and, except as regards muscle and some other fibrillar structures, it appears predominantly in dead secretions and enclosures rather than in the living protoplasm. It has been pointed out repeatedly that the appearance of anisotropy, particularly in various fibrillar structures, may be the result of mechanical tension. Very generally the axes indicated by such anisotropy have reference to local conditions, single cells or fibrils, etc., but in some of the unicellular organisms the anisotropy of the ectoplasm, or the outer layers of it, indicates a close relation between the axis of the organism and the structural system underlying the anisotropy. There is nothing in the evidence to justify the assumption that anisotropy is a primary, inherent property of protoplasm. Apparently it arises

[2] See, for example, VALENTIN 1861, 1871 a, b; ENGELMANN 1875, and references given by these authors. PFEFFER (1897, p. 70) gives references to the more important botanical work along this line.

secondarily, either because of the crystalline character of the substance in which it appears, in consequence of mechanical tension, or possibly of other local conditions. These investigations, then, afford no evidence in support of the molecular or micellar theories of polarity and symmetry.

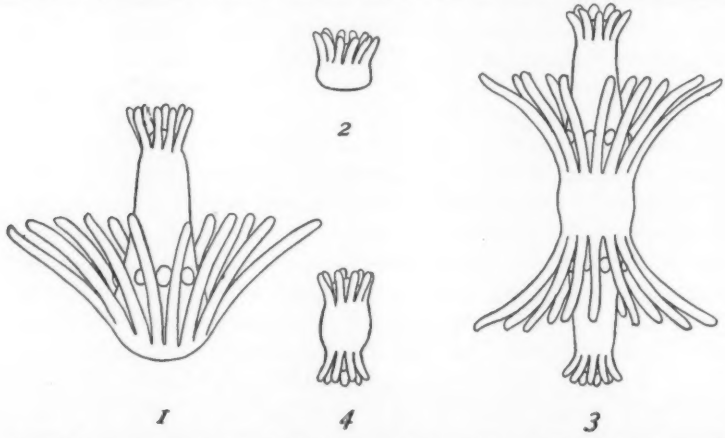
Molar predeterministic theories of polarity and symmetry, e.g., hypotheses of inherent stratifications or directions of flow of substances or inherent morphological structure of protoplasm have been found inadequate to account for the facts of reconstitution of isolated pieces of organisms and have been discarded by most biologists.

If polarity and symmetry are not inherent properties of protoplasm, it is evident that in the final analysis they must be in some way impressed upon protoplasm from without, though the possibility exists that, once established, they may persist in many protoplasms, even through the processes of cellular or individual reproduction. When we turn from speculative hypothesis to the data of observation and physiological experiment, we find first, that polarity and symmetry in their simplest known terms are quantitative gradations in physiological condition, indicated by gradients in rate of respiration, permeability, electric potential, susceptibility, rate of development, and often visible structure; second, that such gradients, and with them polarity and symmetry, can be experimentally established, modified and obliterated in protoplasms by the action of quantitative differentials in dynamic environmental factors; and third, that the localization and differentiation of organs along an axis depend upon the existence of such gradients and can be determined, modified or inhibited as the gradients are determined, modified, or obliterated. The evidence for these statements is on record in various publications[3] and the present paper is primarily concerned, not with this evidence, but with the question of the relation between the physiological gradients and hereditary constitution. Even though we accept all the evidence for the existence of the physiological gradients, we must ask the question whether the gradients may be primarily the expression of an inherent molecular, or other organization which is merely subject to modification by environmental differentials. Certain experimental data seem to me to be of almost crucial importance in this connection, since they leave little or no reasonable ground for the assumptions of an inherent polarity. In following sections some new data are briefly presented and attention is called to other data of importance which have been presented elsewhere.

[3] See CHILD 1915, 1921 a, and references there given.

PARTIAL AND MULTIPLE AXES IN THE RECONSTITUTION OF PIECES OF CORYMORPHA

Corymorpha palma, a tubularian hydroid of the southern California coast, in which the unbranched stem is naked over most of its length, constitutes material of great interest for the investigation of polarity in relation to environmental conditions.[4] While the course of reconstitution in this species is in many respects similar to that in Tubularia, certain differences result from the absence of the perisarc in Corymorpha. The stem often attains a length of 10 to 12 centimeters and the shorter pieces, down to two millimeters or even less, show a great variety of axial relations in reconstitution, the forms which develop being dependent on various conditions and a considerable degree of experimental control being possible. As in Tubularia, the single and biaxial apical structures are of frequent occurrence. The single forms may range from complete individuals through hydranths without stems (figure 1) to structures representing



only the extreme apical end of the manubrium (figure 2), with all possible intermediate forms between these extremes. In all such forms the structure is apparently complete and normal from the apical end basipetally, so far as it is present at all, and the basal ends of such structures terminate abruptly at one axial level or another without any indication of more basal parts, and such parts do not develop later.

[4] I wish to express my appreciation of the kindness of the Director and Staff of the SCRIPPS INSTITUTION FOR BIOLOGICAL RESEARCH in providing a research room and all facilities for work during the spring and summer of 1922. An opportunity was thus afforded me to confirm and extend the results of investigation on Corymorpha which was begun some seventeen years ago.

The biaxial forms also show all possible graduations from complete hydranths with a stem region between them, through biaxial hydranths without stem (figure 3), to biaxial structures including only the distal tentacles and mouth region (figure 4), and occasionally, biaxial hypostomes without even distal tentacles have been observed. In these, as in the single forms, structure is apparently complete and normal from the apical end basipetally, so far as present at all.

As regards the origin of these single and biaxial forms from short stem pieces, we find that in the single forms either the apical or the basal cut

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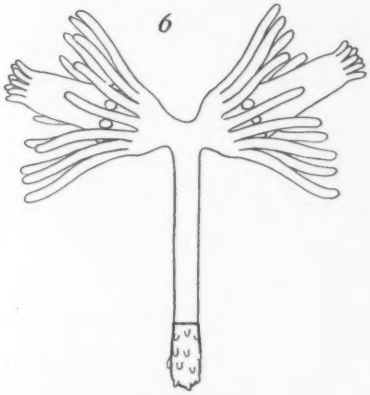

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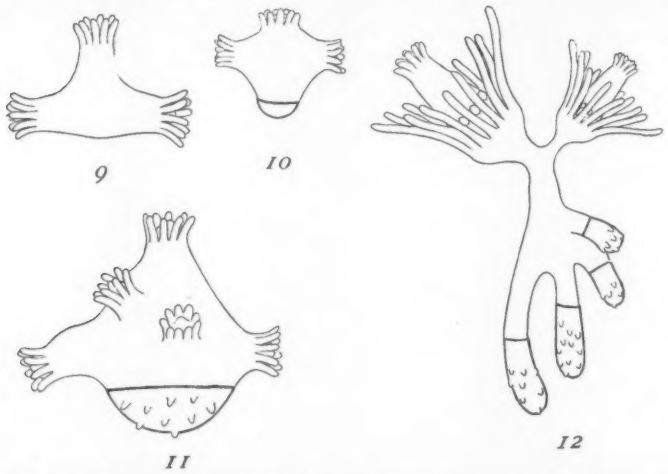
surface usually forms the new apical end according to the position of the piece in the dish, i.e., the cut surface which is exposed to the water becomes the apical end, that in contact with the substratum, the basal end of the structure which develops. In the biaxial forms the two cut surfaces usually become the two apical ends.

In a larger proportion of the short pieces, however, axial relations are further complicated by a great variety of combinations of biaxial, or even multiaxial, and uniaxial structure. A few such forms are shown in

figures 5 to 8. Figure 5 shows indications of a uniaxial manubrial region connecting the two hypostomes. In figure 6 two manubria are connected with a single proximal tentacle region, but without stem. Figure 7 shows a case of almost complete biaxial hydranths with uniaxial basal end, inclosed in perisarc and showing frustule outgrowths, in place of the proximal tentacles on one side and without any trace of stem between the hydranth and the basal structure. Figure 8 is a case of complete biaxial hydranths with uniaxial stem and base.

In forms of this sort the apical ends of the biaxial portions usually, if not always, develop at the two cut surfaces and the uniaxial basal structure, whether it be a certain level of the manubrium (figure 5), a hydranth base (figure 6), or a more or less complete stem and holdfast region



(figure 7 and 8), arises from that side of the piece which lies in contact with the substratum during the earlier stages of the reconstitution.

Not infrequently also cases of multiple partial polarities appear which involve the origin, either of more than one axis on a cut surface, or of "adventitious" axes arising as buds independently of the cut surfaces (figures 9 to 12). Figure 9 consists of three apical ends only, figure 10 of three apical ends and one basal end without trace of intervening regions, figure 11 of five apical ends and one basal end, also without trace of intervening parts, and figure 12 of unequal biaxial hydranths with four basal ends. Numerous other multiple combinations might be mentioned.

It is possible to control the frequency of these different forms to a considerable degree. For example, short pieces which rest on one cut surface during the earlier stages of reconstitution usually, perhaps always, develop apical structures on the free surface and some degree of basality on the surface in contact. Figure 13 represents an early stage of such a piece; the portion of the axis represented varies with length of piece, level of stem from which the piece is taken, and probably also with length of time it remains in the same position, though this point has not been investigated. Short pieces which lie on their sides and are frequently moved about so that any particular region of the lateral surface does not remain in contact for a long period, also pieces which are supported well above the bottom of the dish on very loose absorbent cotton, give a high frequency of purely biaxial forms like figures 3 and 4. On the other hand, pieces which are left undisturbed on their sides on the bottom of the dish, usually show some combination of biaxial and uniaxial structure (figures 5 to 8). As already noted, the apical ends of the biaxial portions are usually localized at the two cut surfaces and the uniaxial portion arises from the region

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in contact with the bottom. Pieces of relatively large diameter, commonly those from the more basal levels of the stem in which the diameter may be three or four times as great as at distal levels, are the usual sources of the multiaxial forms. On the free surfaces of such pieces several apical ends may arise (figure 14, early stage; figures 10, 11, more advanced), and on the cut surface in contact, one or more basal structures (figures 10 to 12).

In general then the factors determining axial relations in these pieces are: (1) the cut surfaces; (2) certain differences between free surface and surface in contact with the bottom; (3) "adventitious" budding on large exposed surfaces, usually cut surfaces, i.e., apparently a localization of axes by fortuitous differences of some sort.

As regards the possibility of interpretation of these varieties of axial relation in terms of an inherent molecular or micellar structure, only a brief consideration seems necessary. Doubtless such interpretation is

possible, but it is possible only with the aid of a number of special assumptions for which there is no evidence. In the first place special assumptions are necessary to account for the appearance in any case of partial axes, since, according to theory, the whole organization is present in every cell and therefore in all multicellular pieces. And even if we assume that the piece possesses a lesser degree of polarity than the whole, we should expect that the piece would give rise to the middle regions of the axis, rather than to one or both of the terminal regions. Assumptions concerning localization of direction of flow of "formative" or other substances will not help us out, because partial and multiple polarities may, by proper procedure, be produced from any level of the body.

Again, axes arise in relation to cut surfaces, but we have not the slightest evidence that such regions exercise an orienting influence on the molecules, particles or cells of the piece in general. Similarly, the determination of axes between free surfaces and surfaces in contact requires further special assumptions concerning the orientation of molecules, particles or cells, for which we have no evidence. And finally, the various combinations of multiple and single polarities (figures 5 to 8 and 10 to 12) must involve very considerable departures from the normal molecular or micellar structure and orientation, at least in certain regions where the various partial axes come together. In figure 7, for example, the two hydranths are in part bilaterally, in part radially symmetrical. To account for such combination on a molecular or micellar basis, we must assume considerable and definite and orderly changes in constitution of the molecules or micellae, and there is no reason to believe that the conditions of the experiments can bring about such changes. Radial symmetry, for example, is a fundamental characteristic of Corymorpha pattern, yet it is possible by controlling the position of pieces to obtain various sorts of bilateral structures, but, except for the bilateral order, such structures still show the characteristics of Corymorpha. Evidently the polarity and symmetry relations can be fundamentally changed without changing the protoplasm from Corymorpha protoplasm into something else. It is highly improbable at least that the molecular changes necessary to produce such fundamental changes in general pattern could take place without affecting in some way the more specific details of pattern. As a matter of fact, we should expect that such alterations in the fundamental molecular substratum would affect the pattern down to its minute details, but there is not the slightest indication that this is the case. In short, attempts to interpret these cases in terms of molecular or other inherent protoplasmic structure involve us at once in

highly speculative assumptions and hypotheses, which are totally unsupported by any evidence.

On the other hand, the interpretation of these axial modifications in terms of physiological gradients is simple and is based on extensive experimental evidence from many different fields, rather than on assumption. As a matter of fact, we find experimentally that wherever in these pieces a region of high metabolic rate arises and persists for a certain length of time, varying with the character of the protoplasm and the conditions, a physiological gradient is initiated, i.e., a quantitative gradation in physiological condition from the region of highest rate to surrounding parts arises. When such a gradation is once established, the hereditary constitution of the protoplasm in which it appears determines what it shall become. No matter what the particular factor by which it was determined, or whether it was originally steep or slight, long or short, if it is once impressed upon the protoplasm so as to persist, the nature of the protoplasm determines the final result. In short, the environmental or physiological conditions give rise to the differential which initiates the gradient, but hereditary constitution determines its later characteristics. As I have pointed out repeatedly, experiment shows further that such a gradient is a physiological axis in its simplest known terms and that the different levels of such a gradient are effective in localization and differentiation of organs. In the polar gradient, for example, apical or anterior ends develop from the high end, basal or posterior ends, from the low end of the gradient and intermediate parts from intermediate levels.

In the short Corymorpha pieces little remains of the original gradient, consequently the two ends of the piece are almost alike physiologically, and each cut surface becomes a region of high metabolic rate because of the wound reaction and growth there. Each such surface may then determine a gradient. One of these gradients reinforces and modifies what remains of the original gradient in the piece, the other is opposed in direction to the original gradient. If external conditions are essentially alike as regards the two cut surfaces, both gradients may determine axes with apical ends at the cut surfaces (figures 3 and 4); if the original gradient is sufficiently reinforced by the new gradient from the apical cut surface, or if external conditions determine a differential which tends to reinforce or to obliterate either of the two opposed gradients, one gradient may determine a single axis throughout the length of the piece and a single structure results (figures 1 and 2).

In the case of Corymorpha the environmental differential between

the free surface and the surface in contact may also determine polarity. Various facts, which need not be discussed here, suggest that this differential involves chiefly oxygen or CO_2 or both, though other factors may also be concerned. Oxygen is much more accessible and CO_2 is more readily given off at the free surface than on the surface in contact. Consequently the free surface may attain a higher rate of oxidative metabolism than the surface in contact and in this way a physiological gradient may be established. But so long as the lower surface remains in contact, its rate may be directly determined by the conditions to which it is subjected. If these determine a sufficiently low rate it may give rise to an extreme basal end, even though extreme apical structures are immediately above it (figures 7, 10 and 11), while in other cases the surface in contact may give rise to the basal region of a hydranth (figure 6), or even to some portion of a manubrium. In short, conditions on the surface in contact do not determine any particular structure, but merely some degree of basality, as compared with the free surface.

The "adventitious" axes apparently originate from areas of the cut surface, or of other regions in which metabolism is slightly higher than in surrounding parts, so that new gradients are gradually determined. The irregular arrangement of such axes in Corymorpha indicates that they are "fortuitously" localized, but I have shown for Harenactis that such axes may be experimentally localized by localizing a greater degree of injury and so of more rapid metabolism and growth (CHILD 1910). It is scarcely to be expected that metabolic rate should be exactly the same at all points of the cut surface of a Corymorpha stem, particularly if of larger diameter, and if differences are present, they may determine new axes.

The frustules at the basal end of the stem are modified stolons and each one has been shown to be a gradient, arising secondarily by a process of budding. Conditions determining metabolic rate of the surface in contact may localize this process in various ways, so that a single, or several basal ends may arise (figures 7, 10, 11 and 12).

OTHER SIGNIFICANT DATA

While the evidence from the short pieces of Corymorpha is particularly striking as regards the various combinations of different factors in giving rise to physiological gradients and so to axes, many other experimental data obtained from other forms present essentially the same difficulties to molecular or other theories of inherent structural polarity and symmetry. For example, single and biaxial partial forms like those of Cory-

morpha (figures 1 to 4) and presenting the same problems, have long been known in Tubularia and have been discussed by BICKFORD, DRIESCH, MORGAN and myself. Since the Tubularia stems are inclosed in perisarc the differentials at the two cut ends are the chief factors in determining new polarities and the various combinations of axes described for Corymorpha do not appear.

In Planaria also single tailless and headless and biaxial heads and biaxial tails have long been known. These partial axes raise the same questions as those in the hydroids. Moreover, it has been shown that the frequency of headless forms and of complete axes can be altered by temperature, age, nutrition, and many different chemical agents (BEHRE 1918; BUCHANAN 1922; CHILD 1916a, 1920) which can be shown to alter the gradients, but which can scarcely be conceived as altering structure and orientation of molecules or particles of the protoplasm.

Again, the apical regions of various colonial hydroids can be transformed into stolons by low concentrations of acids, various salts, anesthetics, standing water, etc. (unpublished). Also in long pieces of Corymorpha stems the old polarity may be obliterated by chemical agents because of the differential susceptibility of different levels of the gradient, and in its absence the differential between free surface and surface in contact determines a new axis (CHILD 1915, pp. 142-146). And finally, the differential susceptibility of different levels of the physiological gradients has made it possible to produce experimentally extensive modifications of polarity and symmetry in two opposite directions by means of many different agents and conditions in various forms (CHILD 1916b; BELLAMY 1919, 1922). In general these modifications are non-specific to a high degree, i.e., similar in character for many different agents and in two opposite directions, the one resulting from differential inhibition and showing all degrees of obliteration of symmetry and polarity with spherical symmetry or surface-interior pattern as the extreme case; the other resulting from differential acclimation and differential recovery and showing increase of the axial differences. The different head forms of Planaria may also be mentioned here as differential inhibitions, acclimation or recoveries, chiefly in the symmetry gradients (CHILD 1921 b), and cyclopia in fishes and other forms belongs in the same category. As shown in the papers referred to, these modifications may be readily and simply interpreted and accounted for in terms of physiological gradients, but I do not believe they can be adequately accounted for in terms of inherent molecular or other structure and orientation, and any attempt at interpretation in such terms requires the aid of highly im-

probable assumptions. All the facts indicate that the underlying conditions determining these various modifications of pattern are primarily quantitative and metabolic in character.

CONCLUSION

If it is true that polarity and symmetry in their simplest terms are quantitative physiological gradients in protoplasms, which are determined in the final analysis directly or indirectly by a differential action of some factor external to the protoplasm concerned, it follows that the physiological radius or axis represents a reaction to environment of a specific protoplasm. In other words, the physiological gradient is the result of the reaction to the environmental factor, but the nature of the organs and parts which arise at the different levels of a gradient is determined by the hereditary constitution of the protoplasm. These organs and parts then represent reactions of the specific protoplasm to the presence of the gradient. This viewpoint by no means excludes the possibility that a gradient, once established, may persist through reproductive processes and so be hereditary for a later individual or generation. Certainly in many such cases, very probably in all, inheritance is cytoplasmic, i.e., the gradient simply persists in the cell or cell mass as it does in many cases of agamic reproduction. This may be the case also in some eggs, while in others the differential to which the growing oöcyte is exposed in the ovary may determine polarity, and conditions connected with fertilization, union of pronuclei, the first cleavage spindle, or some other factor, may determine bilaterality.

In a physiological sense then the individual organism as an order or pattern represents a reaction to environment of a specific protoplasm. This reaction provides the physiological differential which in turn determines the realization in development of different hereditary potentialities in different cells or cell groups. This conception of the individual and of its development as a reaction of a specific germ plasm to an environmental differential, is not only not in conflict with current theories of heredity, but is made necessary by them, since they provide no basis for localization and differentiation of organs and parts. Many different lines of experimental evidence force the conclusion that this reaction in its simplest terms is a quantitative gradient, involving the fundamental metabolic reactions and associated protoplasmic conditions.

SUMMARY

Theories of physiological polarity and symmetry based on assumptions of inherent structure and orientation of molecules or other particles in

protoplasm are not supported by current conceptions of heredity nor by experimental evidence. The facts indicate that polarity and symmetry originate as physiological reactions of specific protoplasms to differentials of some sort in the environment of the protoplasm concerned. These reactions give rise to gradations in physiological condition, the physiological gradients, which provide a physiological basis for the realization of different hereditary potentialities in different regions, cells, or cell groups. The character of the reaction gradient and of the organization to which it gives rise in each particular case depends of course, not upon the environmental differential, but upon the hereditary constitution of the protoplasm concerned.

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AN EFFECT OF X RAYS ON THE LINKAGE OF MENDELIAN CHARACTERS IN THE FIRST CHROMOSOME OF DROSOPHILA

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INTRODUCTION

In the course of experiments designed to test the effects of X rays in causing non-disjunction of the sex chromosomes of Drosophila (MAVOR 1921 a, b, 1922, 1923) opportunity was given also to test for an effect of X rays on crossing over. Crossing over in the sex chromosomes of Drosophila has been studied in great detail by MORGAN and BRIDGES (1916) and others. The very extensive data collected by these investigators have been used to construct chromosome maps and to develop subsidiary theories of the mechanism of crossing over. BRIDGES (1915) has shown that the amount of crossing over in the second chromosome decreases with the age of the fly. PLOUGH (1917) found that temperatures above and below the normal breeding temperature (22°C) caused an increase in the amount of crossing over in the second chromosome. Further investigation of the temperature effect by the same writer (PLOUGH 1921) showed that crossing over in a region of the third chromosome was increased by exposure to a temperature above normal. He found, however, "that a temperature of 31.5°C causes little or no effect on crossing over in any part of the sex chromosome, nor is there any significant variation with the age of the female." DETLEFSEN and ROBERTS (1921), using the recessive characters white eye and miniature wing (the same as those used in the present investigation), have found that crossing over in the sex chromosome can be decreased by breeding from individuals showing a low crossover value. They were however unable to increase the crossover value by selection in the reverse direction.

When a white-eyed, long-winged female is crossed with an eosin-eyed, miniature-winged male, the daughters are all heterozygous and may be represented by the formula $\frac{w\ M}{w^e\ m}$, indicating that one of the X chromosomes carries the determiners for white eye color (w) and long wings (M), while the other carries the determiners for eosin eye color (w^e) and miniature wings (m). If such a heterozygous female is bred, she will have four kinds of regular sons irrespective of the male with which she is crossed, since the regular sons obtain their X chromosomes only from their mother. In two of the kinds of sons the characters will appear as they entered in the original cross, i.e., one kind will be white-eyed and long-winged and the other eosin-eyed and miniature-winged; these make up the non-crossover classes. In the other two kinds, on the other hand, the characters will be interchanged, i.e., one kind will be white-eyed and miniature-winged and the other eosin-eyed and long-winged; these make up the crossover classes. It is usual in work on crossing over to cross the heterozygous individual,—in this case a female,—with a double recessive, which in this case would be a white-eyed, miniature-winged male, so that the daughters also show the four classes described above. The experiments to be described were designed primarily to test for non-disjunction and for that reason the heterozygous females were mated to males with the dominant character, red eyes, so that the exceptional sons and daughters could be recognized. This did not of course affect the characters of the regular sons and the data presented give the crossover values obtained from counts of these. No attempt has been made to correct the data for double crossing over.

Effects due to differences in the viability of the classes have not been entirely eliminated. To do this would involve repeating the experiment using for the control and X-rayed females, flies of the formula $\frac{w\ m}{w^e\ M}$. However, it will be noted that each of the mutations occurs in one of the crossover and one of the non-crossover classes, and further, that in neither the offspring of the control nor of the X-rayed females is there a significant difference between the classes of crossovers or between the classes of non-crossovers.

The evidence for the effect of X rays on crossing over rests entirely on a comparison of the crossover values for the X-rayed and control females, as must be the case in any investigation of the effect of an external agent. In the experiments, both the X-rayed and the control females were sisters which emerged at approximately the same time, were kept at the

same temperature (23°C) in bottles of the same size containing banana agar made at the same time from the same bananas and sprayed with yeast from the same cake. On account of not being corrected for double crossing over the crossover values given in the tables for the controls are lower than those usually given for the characters concerned.

X-RAY EXPERIMENTS

A brief statement may be made concerning the X-ray technique and the method of recording the dose. A water-cooled Coolidge X-ray tube with tungsten target was used. It was operated at 50,000 volts, root mean square, alternating current. The number of milliamperes passing through the tube, the distance from the target and the time of treatment were different in the different experiments. The following method has been used to express the doses in comparable form. A dose in which the number of milliamperes was 1, the distance from the target 10 cm and the time 1 minute, was taken as a unit and called D. Any dose at 50,000 volts is therefore expressed by $\frac{\text{number of milliamperes} \times \text{time in minutes}}{(\text{distance in decimeters})^2}$.

The first experiments in which the effect of X rays on crossing over was tested were those of the third series of X-ray experiments. The females, $\frac{w\ M}{w^s\ m}$, were obtained virgin by isolating pupae in test tubes containing banana agar. The X-ray dose was given to the females before mating. This dose, which was always at 50,000 volts, varied in the third series of experiments from 21 to 49 D. The females were mated, immediately after being X-rayed, to wild-type males. The pairs, both X-rayed and control, were allowed to remain in the first bottles for six days and in the second bottles for eight days. The F_1 were counted in the bottles until eighteen days after the parents were placed in the bottles, the temperature of the incubator being maintained between 22.7° and 24.4° C.

The results of the counts for the two sets of bottles and the different doses of X rays are given in table 1 which also gives the differences between the number of crossovers in the F_1 of the X-rayed and the mean number to be expected from the controls divided by the probable error of the difference.[1] This table shows that there is no significant change in the crossover value in the first bottles of the X-rayed. This is true for the totals corresponding to the different doses of X rays and it is also true for the total of all the experiments in the series $\left(\frac{\text{Diff.}}{\text{P.E.}_{\text{diff.}}} = .87\right)$.

[1] For the formula used in making these calculations see K. PEARSON (1907).

TABLE 1

Crossover values. Third series of experiments.

First bottles

Dose in D	X-RAYED						CONTROL						DIFF.
	Number of pairs	Non-crossovers		Crossovers		Percent crossing over	Number of pairs	Non-crossovers		Crossovers		Percent crossing over	P.E.*diff.* Control and X-rayed
		w M	*w*^{e} *m*	*w m*	*w*^{e} *M*			*w M*	*w*^{e} *m*	*w m*	*w*^{e} *M*		
21–23	9	57	60	15	19	22.5	9	209	195	95	75	29.7	2.55
26–29	18	99	98	29	43	26.8	20	416	351	128	144	26.1	.27
35–37	19	38	41	21	16	31.9	21	435	372	153	165	28.3	.27
38–43	8	10	6	2	2	35.7	11	218	193	67	75	25.7	.89
44–49	13	23	24	6	9	23.8	16	318	277	108	112	27.1	.16
21–49	67	227	229	73	89	26.2	77	1596	1388	551	571	27.3	.87
Second bottles													
21–23	6	165	143	28	17	12.7	9	164	143	80	60	31.3	8.56
26–29	14	129	112	23	25	16.6	20	279	251	118	111	30.1	8.44
35–37	14	126	114	7	10	7.1	21	336	303	135	149	31.1	11.78
38–43	3	6	4	0	1	9.1	11	160	143	44	58	25.1	1.81
44–49	6	8	16	1	0	4.2	16	314	292	90	124	26.1	3.64
21–49	43	434	389	59	53	12.0	77	1253	1132	467	502	28.9	14.97

On the other hand, in the case of the second bottles there is a significant decrease in the crossover value wherever a sufficient number of F_1 were obtained from the X-rayed females to give a significant result, i.e., after doses of 21 to 23, 26 to 29, 35 to 37 D. After doses of 38 to 43 and 44 to 49 D the number of F_1 obtained from the X-rayed females was so small that little significance can be attached to the crossover values obtained. The total for all the experiments of the series (dose 21 to 49) shows also a significant decrease in the crossover value both when compared with the control value $\left(\frac{\text{Diff.}}{\text{P. E.}_{\text{diff.}}} = 14.97\right)$ and when compared with the crossover value obtained for the same pairs in the first bottles $\left(\frac{\text{Diff.}}{\text{P. E. }_{\text{diff.}}} = 9.31\right)$. The table also indicates that the crossover values in the second bottles decrease as the X-ray dose increases, as may be seen by comparing the crossover values for doses of 21 to 29 D and 35 to 49 D, when the difference is found to be 4.8 times the probable error of the difference.

In the fourth series of experiments the parent flies were of the formula $\frac{w\ M}{w^e\ m}$ and the experiments were conducted in exactly the same way except for three important differences. The females used in the experiments, both for the control and X-rayed, were the offspring of one white-eyed female. The X-ray dose in this series was that found in the third series to be the most suitable for modifying the crossover value, namely, 35 to 38 D. The time of treatment was varied between wide limits. Group I, which received a dose of 35 D, was treated for 3 minutes and 17 seconds; group II, which received 38 D was treated for 2 hours and 15 minutes; and group III, which received 36 D, was treated for 20 hours and 20 minutes. Group III, therefore, while receiving only 2.9 percent more radiant energy, $\frac{(\text{milliamperes} \times \text{time})}{\text{distance}^2}$, than group I, received that energy during a time which was 372 times as long (20 hours and 20 minutes) as the time during which the flies of group I received their X-ray dose. The X-rayed and control females were transferred to new bottles every three days, so that the first and second bottles of the fourth series of experiments together correspond to the first bottles of the third series, and the third and fourth bottles of the fourth series to the second bottles of the third series. The offspring were counted until eighteen days after the parents were placed in the bottles.

TABLE 2

Crossover values. Fourth series of experiments.

First bottles

GROUP	X-RAYED						CONTROL						DIFF. P.E.diff. Control and X-rayed
	Number of pairs	Non-crossovers		Crossovers		Percent crossing over	Number of pairs	Non-crossovers		Crossovers		Percent crossing over	
		$\frac{w\ M}{}$	$\frac{w^e\ m}{}$	$\frac{w\ m}{}$	$\frac{w^e\ M}{}$			$\frac{w\ M}{}$	$\frac{w^e\ m}{}$	$\frac{w\ m}{}$	$\frac{w^e\ M}{}$		
I	11	54	49	16	23	27.7	9	181	170	75	81	30.8	
II	8	23	22	6	10	26.3	6	167	171	68	70	29.0	
III	7	26	29	10	11	27.6	4	72	82	29	35	29.4	
I–III	26	103	100	32	44	27.2	19	420	423	172	186	29.8	1.27
Second bottles													
I	10	49	46	14	16	24.0	9	266	230	113	103	30.4	
II	7	20	21	8	7	26.8	5	169	145	80	78	33.5	
III	7	18	19	8	6	27.5	4	96	106	57	35	31.3	
I–III	24	87	86	30	29	25.4	18	531	481	250	216	31.5	2.77
Third bottles													
I	8	158	136	13	8	6.7	9	212	221	66	83	25.6	
II	7	87	88	13	10	11.6	5	164	139	49	56	25.7	
III	6	78	77	11	9	11.4	4	119	103	49	59	32.7	
I–III	21	323	301	37	27	9.32	18	495	463	164	198	27.4	12.85
Fourth bottles													
I	9	165	183	23	14	9.6	9	209	228	70	95	25.8	
II	7	55	62	10	7	12.7	5	85	115	54	40	32.0	
III	6	110	97	8	11	8.4	4	73	88	38	25	28.1	
I–III	22	330	342	41	32	9.80	18	367	431	162	160	28.7	13.16
First and second bottles													
I		103	95	30	39	25.8		447	400	188	184	30.5	1.68
II		43	43	14	17	26.5		336	316	148	148	31.2	1.56
III		44	48	18	17	27.6		168	188	86	70	30.5	.97
I–III		190	186	62	73	26.4		951	904	422	402	30.8	2.90
Third and fourth bottles													
I		323	319	36	22	8.3		421	449	136	178	26.5	16.98
II		142	150	23	17	12.0		249	254	103	96	28.4	8.08
III		188	174	19	20	9.7		192	191	87	84	30.9	10.43
I–III		653	643	78	59	9.56		862	894	326	358	28.0	18.34

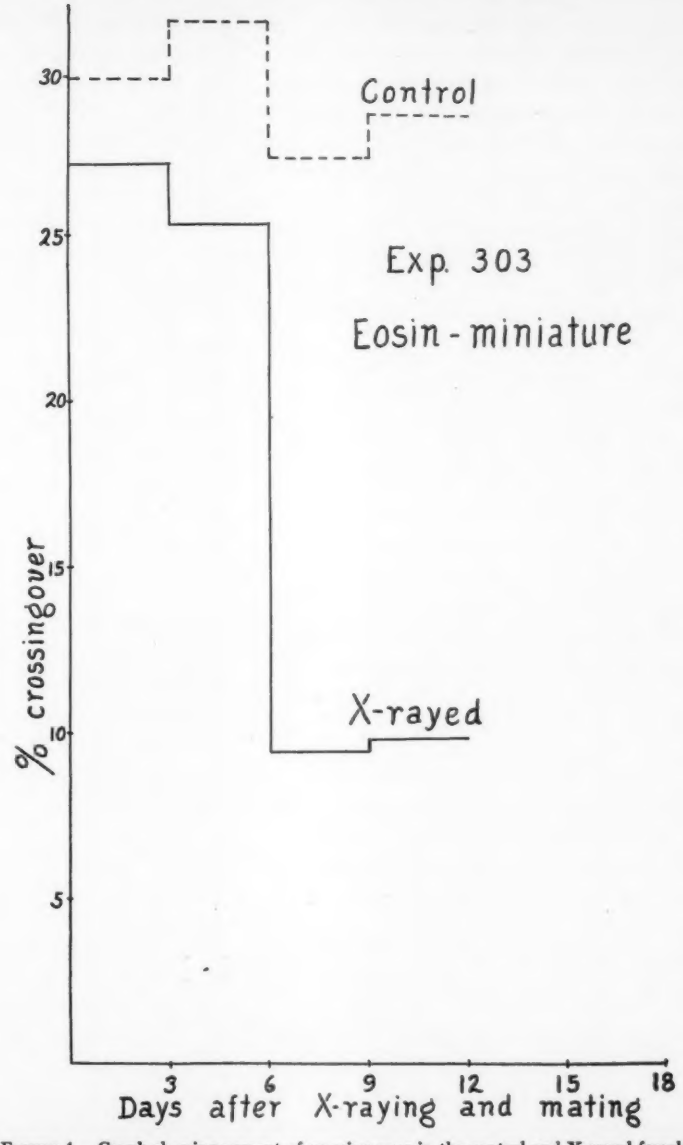

FIGURE 1.—Graph showing percent of crossing over in the control and X-rayed females of experiment 303, fourth series.

The results of the fourth series of experiments are shown in table 2, and graphically in figures 1 and 2. We find here, as in the third series of experiments, that there is no significant difference in the crossover values obtained from the first two bottles (eggs laid during the first six days) of the X-rayed and control flies. For the three groups taken together the difference between the crossover values found for the X-rayed and the mean expected from the controls, divided by the probable error of the difference is for the first bottles (eggs laid during the first three days) 1.27 and for the second bottles (eggs laid during the second three days) 2.77, showing that

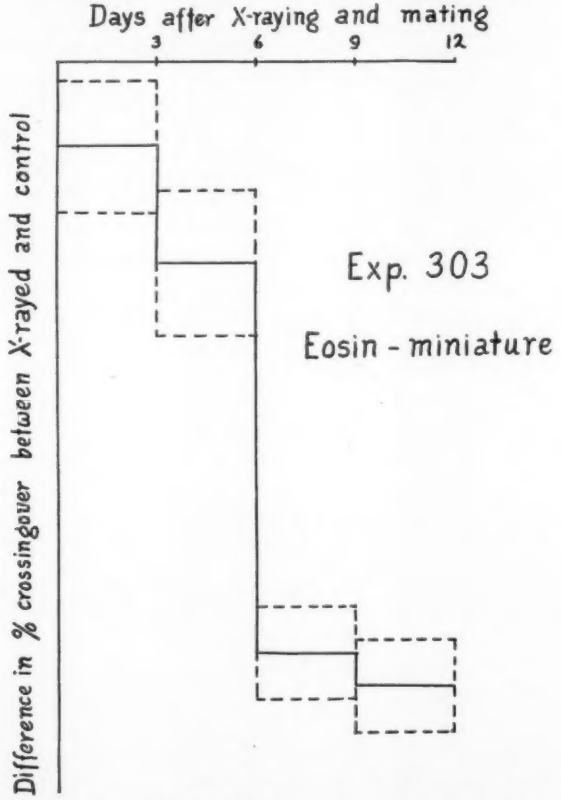

FIGURE 2.—Graph showing the difference between the percent of crossing over in the control and X-rayed females of experiment 303, the difference being indicated by the continuous lines, and the probable error of that difference by the broken lines. The vertical scale has been omitted but is the same as in figure 1.

these differences are probably without significance. In the case of the third and fourth bottles the difference in the crossover values obtained for the X-rayed and control females is clearly seen. For all three groups taken together we find in the third bottles a crossover value of 9.32 percent for the X-rayed females as compared with a crossover value of 27.4 percent for the control females, the difference being in this case 12.85 times the probable error. In the fourth bottles for the same three groups we find a crossover value of 9.80 percent for the X-rayed females and 28.7 percent for the controls, the difference in this case being 13.16 times the probable error. It is thus apparent that the effect of the X rays has been to decrease the crossover value in both the third and fourth bottles. The effect of the X rays thus extends over a period of approximately six days. This is true of group I taken alone, as is shown by the difference between the crossover values for the control and the X-rayed females, being, in the case of the third bottles, 9.62 and in the case of the fourth bottles, 9.16 times the probable error of the difference. We therefore have the somewhat surprising result that an X-ray treatment lasting only 3 minutes and 17 seconds produces an effect on crossing over which is evident for six days, and further that the effect of X rays on crossing over depends within wide limits only on the total radiant energy received by the flies and not on the duration of the treatment. It is also to be noted that in each of the three groups the difference between the crossover value found for the first two bottles of the X-rayed females and that found for the last two bottles of the same females, is significant, the differences being 8.37, 4.66, 5.96, 11.07, times the probable error of the difference in groups I, II, III and I to III, respectively. The table for the fourth series shows more clearly what is also apparent in the table for the third series, namely, that the crossover value becomes modified by the X rays at about the same time as the flies recover their fertility after the partial sterility produced during the first six days by the X-ray treatment. Stated in another way, during the time that the X rays produce an alteration in crossing over in the eggs, few eggs are fatally injured by the X rays. It is unfortunate that the series of experiments was terminated in the fourth bottles and that the control and X-rayed flies were not transferred to fifth and sixth bottles.

The results of the fourth series confirm those of the third series. Since the data are comparable, the females having had the same genetic constitutions, it is possible to add the numbers of non-crossovers and crossovers in the first bottles of the third series to the corresponding numbers for the first and second bottles in the fourth series and similarly to add the

numbers of non-crossovers and crossovers in second bottles of the third series and the third and fourth bottles of the fourth series. When this is done we find that the difference between the crossover values of the control and X-rayed females for eggs laid during the first six days is 2.03 times the probable error and that the difference between the crossover values of the control and X-rayed females for eggs laid during the second six days of egg-laying is 28.37 times the probable error of the difference. The evidence for X rays having an effect on the crossover value is therefore very strong.

Daughters of the X-rayed and control females were bred to determine if the decrease in the crossover value produced by the X rays was inherited. The data which are somewhat meager are given in table 3. There is no evidence of inheritance of the decreased crossover value in either the first or second bottles of the daughters.

TABLE 3

Crossing over in X-rayed females and their daughters.

		X-RAYED		PERCENT CROSSING OVER		CONTROL			PERCENT CROSSING OVER
		Non-cross-overs	Cross-overs				Non-cross-overs	Cross-overs	
X-rayed females 3rd bottles	A′	35	3	7.9	Control females 3rd bottles	A	76	22	22.3
	B′	29	2	6.5		B	64	20	23.6
	C′	29	3	9.4		C	67	24	26.4
Daughters of X-rayed 1st bottles	A′	82	40	32.8	Daughters of control 1st bottles	A	54	24	30.8
	B′	116	39	25.2		B	48	41	45.2
	C′	54	34	38.7		C	39	13	25.0
Daughters of X-rayed 2nd bottles	A′	65	19	22.6	Daughters of control 2nd bottles	A	29	20	40.8
	B′	25	13	34.2		B	27	17	38.6
	C′	18	20	52.7		C	36	17	32.1

DISCUSSION

Were it not clear in the case of the sex chromosome that there is no significant variation in the crossover values with age, it might be objected that the differences in crossover values shown in the data from the experiments could be due to an accelerated development of the germ cells of the X-rayed females, since X rays are known to cause acceleration. This

would mean that the offspring produced by the X-rayed females in the second period when the decreased crossover values were obtained, really corresponded to the offspring produced by older flies than those in the controls. It is of course possible that the development of the germ cells, in the oögonial divisions and maturation stages, is accelerated by the X rays, but such an acceleration would not explain the differences in the crossover values obtained.

In the experiments described no account has been taken of double crossing over. Since the characters used involved only two loci this was not possible. It is therefore possible that the decrease in the crossover value between eosin and miniature may really be due to an increase in double crossing over. Experiments are already in progress using a number of loci in the X chromosome and it is expected that they will give the answer to this question. A difference in the viability of the crossover and non-crossover class accentuated by the X rays may be considered as an explanation of the differences in crossover values obtained. In other words the decrease in the crossover values may be due to a difference in susceptibility between the crossover and non-crossover classes. Since the females were submitted to X rays before mating this would mean that there was a difference in susceptibility between the crossover and non-crossover eggs before fertilization, i.e., before development started—a rather unlikely condition. This suggests another explanation which may be advanced—that the egg is more susceptible to X rays at the time when crossing over is taking place, so that their number is reduced and in consequence the numbers of flies in the crossover classes. In this connection it is to be noted that the decrease in the crossover values was produced by a dose of X rays which lasted 3 minutes and 17 seconds and that the crossover values were reduced from 25.8 percent to 8.3 percent in the eggs laid during six days. Further, practically the same result is obtained if the treatment lasts for 20 hours and 20 minutes. If then the X rays were effective only during the process of crossing over, that process would have to be one of long duration (approaching six days). The results might of course be obtained if the process of crossing over occurred rythmically in the fly, with long intervals, but we know of no facts to support this.

The evidence therefore tends to show that the X rays do not directly affect the process of crossing over but that they produce in the chromosomes or the nucleus generally a condition which inhibits crossing over (or increases double crossing over). If, however, the X rays had a direct

effect on the constitution of the chromosomes one would expect such an effect to be inherited, a result not obtained in the experiments.

From the point of view of the biological effects of X rays two of the results obtained in these experiments are of considerable importance. So far as at present known X rays are the only physical or chemical agents which affect crossing over in the X chromosome of Drosophila. The effect produced by the X rays is independent of the duration of the X-ray treatment over a wide range (factor of 372). This latter result is the same as that obtained when X rays are allowed to act directly on a photographic plate.[2]

CONCLUSIONS

1. It has been shown that X rays cause a decrease in the crossover value between eosin and miniature.
2. The decrease in the crossover value is probably not inherited.
3. An X-ray dose lasting for 3 minutes and 17 seconds produces an effect which is apparent over a period of six days.
4. This suggests that the decrease in the crossover value produced by the X rays is not due to a direct effect of the X rays on the crossing-over process.

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[2] Dr. COOLIDGE tells me that in experiments carried on in the Research Laboratory of the GENERAL ELECTRIC COMPANY no difference could be observed in the effect on photographic plates exposed to intensities represented by 6, .6, .06, .006 milliamperes, when the target distance and the product, milliamperes × time, was kept constant. In this case there was a factor of 1000.

THE INHERITANCE OF SPANGLING IN POULTRY

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INTRODUCTION

The experiments[1] described below were undertaken for the purpose of determining, if possible, the mode of inheritance of so-called "spangling" in the Silver Spangled Hamburg fowl. This breed is characterized by the possession of white feathers each of which is tipped with a definite, sharply demarked, black spot or "spangle." The spangles are, in general, proportional to the size and shape of the feathers and are present in the same condition in both sexes (figures 1, 2 and 3). It was thought that such a clearly cut color pattern would be a favorable one for genetic analysis (LEFEVRE 1917).

The experiments were begun by making reciprocal crosses between Silver Spangled Hamburgs and Brown Leghorns. The latter were obtained from the stock of the Department of Poultry Husbandry of the UNIVERSITY OF MISSOURI, while the Hamburgs, which were acquired by purchase, were found by tests to breed practically true to their characteristic pattern, although DAVENPORT (1909) states that, according to the experience of breeders, the spangled pattern is not reproduced closely.

It has been established by the experiments that spangling is controlled in heredity by a dominant factor that is sex-linked in accordance with

[1] We are deeply grateful to Dean F. B. MUMFORD, Director of the Agricultural Experiment Station of the UNIVERSITY OF MISSOURI, for the liberal grant of money from the funds of the Station, which made these experiments possible.

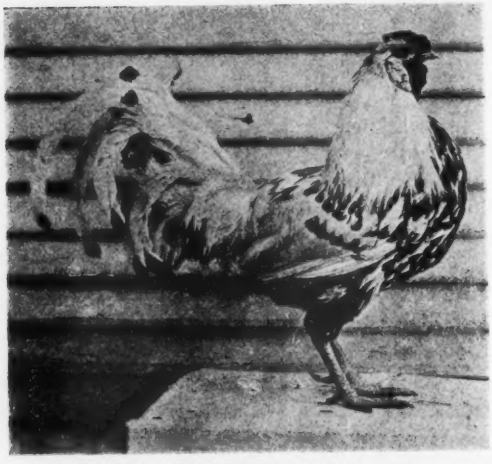


FIGURE 1.—Silver Spangled Hamburg male.

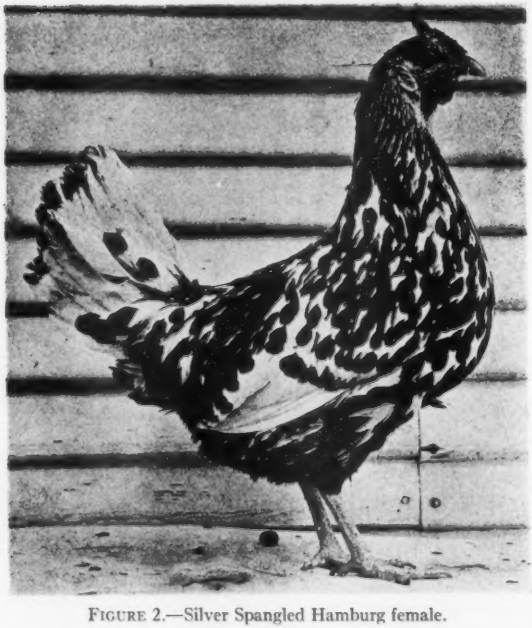


FIGURE 2.—Silver Spangled Hamburg female.

the mode of sex-linkage that is typical of poultry. In the males, therefore, the sex chromosomes may be represented in the conventional manner by ZZ, in the female, by ZW.

It has been shown, furthermore, that the expression of spangling may be greatly modified, or even entirely obscured, by the presence of other factors, especially of factors for black pigmentation, which, however, segregate independently of the factor for spangling and are, therefore, autosomal. These disturbing factors may affect the entire body or only

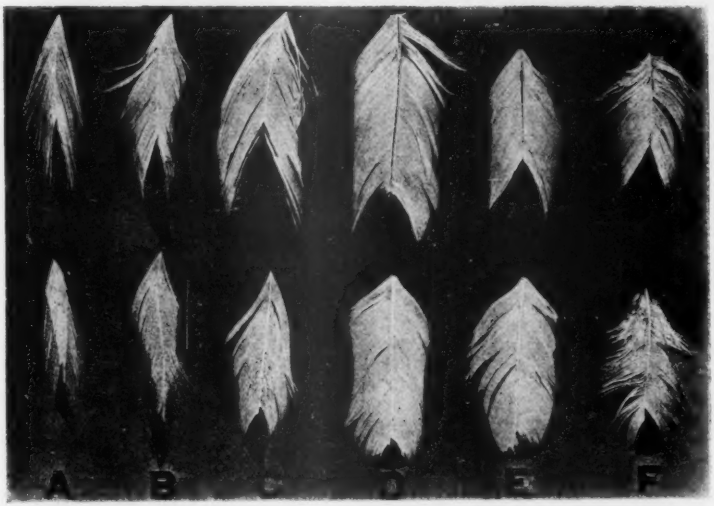


FIGURE 3.—Comparison of spangles on tips of corresponding feathers in the Silver Spangled Hamburg, upper row, and in an individual derived from crossing, in which the spangles are greatly reduced in size, lower row. A, hackle; B, saddle; C, sickles; D, secondaries; E, wing-coverts; F, breast.

some restricted region, like the feathers of the tail. Black pigment may also be present on individual feathers in other places than at the tip, and, in varying degrees, obscure the definiteness of the spangles. In fact, a maximum condition of melanism is sometimes reached, in which black is present to such an extent that the bird becomes self-colored and is practically black all over. In these cases, spangling is of course entirely masked, and the presence of the factor for spangling can only be recognized by making the appropriate breeding tests which demonstrate that these birds transmit spangling without showing it themselves.

It has also been observed that the opposite condition may exist, and that, owing to factors that apparently influence the size of the spangles, the white of the feathers may extend farther toward the tip, thus confining the spangle to a much smaller area. The amount of the area covered by the spangle varies in different individuals, and in some birds the spangle may become so restricted as to appear merely as an attenuated spot, much smaller than the corresponding spangle of the pure Hamburg, or it may be greatly diminished in intensity (figure 3). Just as the presence of black factors may lead to a cumulative effect and thereby cause a varying degree of extension of the pigmentation, it is also true that the restriction of the spangles appears to be cumulative, as different degrees of attenuation are seen.

It should also be mentioned that, although the ground color of the feathers is usually white when spangling is present, it may be replaced by golden bay, a condition seen in the Golden Spangled Hamburgs of the poultry fancier, which, however, is recessive to white and appears only after segregation of the factors concerned in the F_2 generation and later.

A detailed account of the more important matings that have been made and an analysis of the results follow.

MATING 1. BROWN LEGHORN FEMALE WITH SILVER SPANGLED HAMBURG MALE

In the progeny of this mating, spangling appears in both sexes. The cocks show the spangles in a fairly typical fashion throughout the body, except that their tails are glossy black (figure 4). The hens are also spangled and have black tails, but, owing to the presence of more or less black on other parts of the feathers as well as at the tips, the spangling is somewhat obscured. The spangles of the hens are most definite on the neck, back, breast, wing-coverts and secondaries. Figure 5 is a hen from this mating, and although the spangles may be seen distinctly, especially on the neck, breast and wings, a comparison with the cocks shows that the condition is less characteristic of typical spangling.

In addition to these spangled cocks and hens, one male and two females were obtained from the cross that were almost black throughout. Their presence in the brood was at first regarded as inconsistent with the assumption, otherwise borne out, of sex-linkage, but it was later discovered that these black birds, although completely masked by black, transmitted spangling in exactly the same way as did their spangled brothers and sisters. They would, therefore, seem to be best explained by assuming the presence of multiple factors for black which, after segregation

in gametogenesis, have a cumulative effect and produce in a minority of cases birds of both sexes that are practically self-colored.

The mating yielded 22 spangled birds and 3 blacks, a ratio of about 7 to 1.

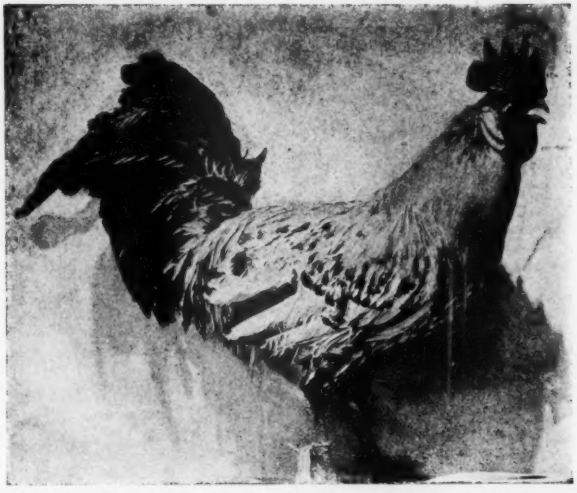


FIGURE 4.—F_1 male from cross between Brown Leghorn hen and Silver Spangled Hamburg cock (mating 1).

The black cock from this cross is shown in figure 6, and the proof of his ability, as well as of that of the black hens, to transmit spangling is presented in mating 6.

As will be indicated below, similar black birds were obtained from the reciprocal cross and from other matings in which only spangled individuals of either one or both sexes were expected, but their occurrence in all such cases may be accounted for on the same assumption of multiple factors for black.

The results of all matings meet with a consistent interpretation on

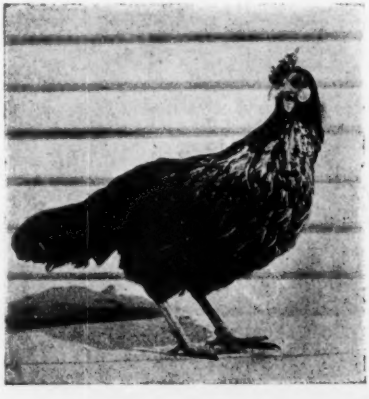


FIGURE 5.—F_1 female from cross between Brown Leghorn hen and Silver Spangled Hamburg cock (mating 1).

the basis of the sex-linkage of a factor for spangling, and, as in all cases of the same kind that have been recorded for poultry, the males may be represented by the formula ZZ, the females by ZW.

The analysis of the results of mating 1 is as follows:

	Males	*Females*
Parents	Z^SZ^S	Z^sW
Gametes	Z^S, Z^S	Z^s, W
F_1	Z^SZ^s	Z^SW

The exponents in the above formulae stand for the sex-linked factors involved, namely, *S* for spangled, *s* for non-spangled.

FIGURE 6.—F_1 black cock from cross between Brown Leghorn female and Silver Spangled Hamburg male, showing full effect of multiple factors for black (mating 1).

The other factors disclosed by the cross need not be here considered. Obviously, there is a dominant black-tail factor received from the Leghorn mother by both sons and daughters, and in a small percentage of the F_1 birds a masking effect of black is seen in the three wholly black individuals already referred to.

MATING 2. SILVER SPANGLED HAMBURG FEMALE WITH BROWN LEGHORN MALE

This is the reciprocal cross of the preceding, and, on the assumption of the sex-linkage of the spangling factor, spangled cocks and non-

spangled hens should be expected. Such was the case. Nineteen spangled cocks with black tails (figure 7), identical in appearance with the cocks from mating 1, were obtained, and three black cocks exactly like the black male (figure 6) from the reciprocal cross also appeared in the brood, the ratio being practically the same as after the former mating, or about 7 to 1.

The hens, on the other hand, were without exception non-spangled (figure 8), being almost self-black, with a little golden brown lacing on the neck.

In the progeny of this cross, we see the typical condition of sex-linkage, since the spangling, when introduced by the female, appears only in the sons, as contrasted with its occurrence in both sons and daughters, when the father is spangled, as in mating 1.

The three black cocks are only an apparent exception, for it has been shown by testing them that they, like the black male from the reciprocal cross, transmit spangling in exactly the same way as do their spangled brothers. In other words, they are heterozygous for the spangling factor, but they themselves fail to show spangles, as they are masked by black throughout.

The analysis of the results of mating 2 is as follows:

	Males	*Females*
Parents	Z^sZ^s	Z^S W
Gametes	Z^s, Z^s	Z^S, W
F_1	Z^sZ^S	Z^sW

FIGURE 7.—F_1 male from cross between Silver Spangled Hamburg hen and Brown Leghorn cock (mating 2).

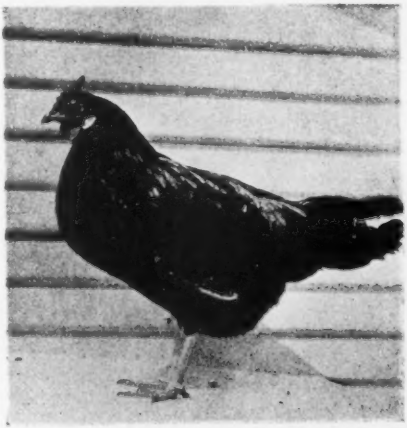


FIGURE 8.—F_1 female (non-spangled) from cross between Silver Spangled Hamburg hen and Brown Leghorn cock (mating 2).

MATING 3. NON-SPANGLED HENS FROM MATING 2 WITH BROWN LEGHORN MALES

It was necessary, in order to prove the sex-linkage of the spangling factor, to show that the non-spangled hens obtained from mating 2 were not only non-spangled in appearance, but that the spangling factor was not received by them and, therefore, could not be transmitted by them. Such a test could be made by mating them to non-spangled cocks, and this was done by breeding several of the hens with pure Brown Leghorn males. Although a large number of birds were obtained from the cross, in not a single instance was there the slightest indication of spang-

FIGURES 9 and 10.—Progeny of F_1 non-spangled hen and Brown Leghorn cock (mating 3).

ling in the offspring. The latter were without exception black with more or less red and yellow streaking. A male and a female from this mating are seen in figures 9 and 10, respectively, in which no trace of spangling is in evidence.

It may, therefore, be concluded that the non-spangled hens of the F_1 generation do not carry a factor for spangling.

The analysis is as follows:

	Males	*Females*
Parents	Z^sZ^s	Z^sW
Gametes	Z^s, Z^s	Z^s, W
Offspring	Z^sZ^s	Z^sW

MATING 4. THE F_2 GENERATION FOLLOWING MATING 1

In order to further demonstrate the sex-linkage of the factor for spangling, matings were made for the purpose of contrasting the results in the F_2 generations derived from the two original crosses, respectively.

It has been seen that in mating 1 spangling appears in both sexes. These spangled F_1 cocks and hens were accordingly mated with the expectation that they would produce only spangled sons and that fifty percent of the daughters would be spangled and fifty percent would be non-spangled. The actual results conformed perfectly to the prediction. Twelve males were obtained, all of which were spangled except one which

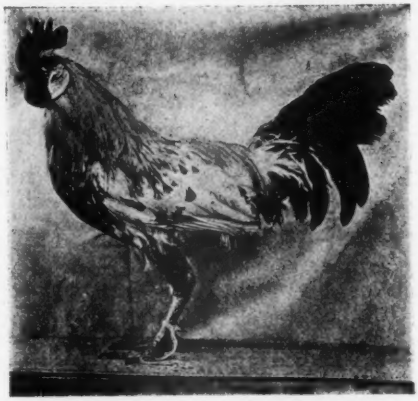


FIGURE 11.—Cock of F_2 generation after cross between Leghorn female and Hamburg male (mating 4).

was identical in appearance with the black cocks from matings 1 and 2 (figure 6) and which again can be accounted for on the assumption of an accumulation of multiple factors for black. Unfortunately, this bird died before it was possible to test him. A majority of the males had black tails and were exactly like the F_1 cocks. Figure 11 shows such a male. Owing, however, to independent segregation and recombination of factors for black and for white, the F_2 cocks exhibited different degrees of the extension of black and white on the feathers. Contrast the male of figure 11 with the one shown in figure 12, which is from the same mating, but which has an excess of black pigment, especially on the under parts, more or less obscuring the spangles. As an illustration of a bird in which the white is extended, observe the condition of the cock seen in figure 13,

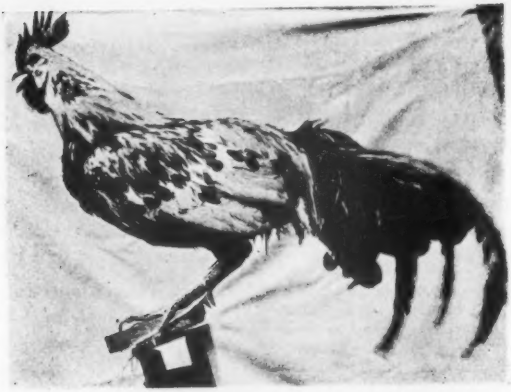


FIGURE 12.—F_2 cock from same mating as figure 11, showing excess of black.

FIGURE 13.—Cock from mating 4, showing reduction of spangles both in size and brilliancy. Some of the spangles are mere shadows and are almost invisible in the photograph.

also from this mating, in which the spangles are much reduced both in size and brilliancy and in some cases appear almost like shadows.

The hens, on the other hand, were of both types, spangled and non-spangled, eight of each having been obtained from the mating, in exact

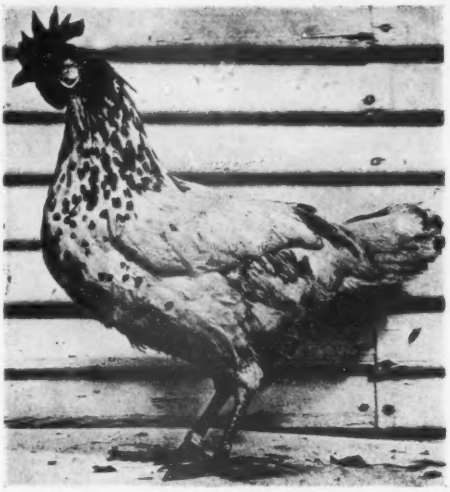


FIGURE 14.—Spangled hen of F_2 generation after cross between Leghorn female and Hamburg male (mating 4).

FIGURE 15.—Non-spangled F_2 hen after cross between Leghorn female and Hamburg male (mating 4).

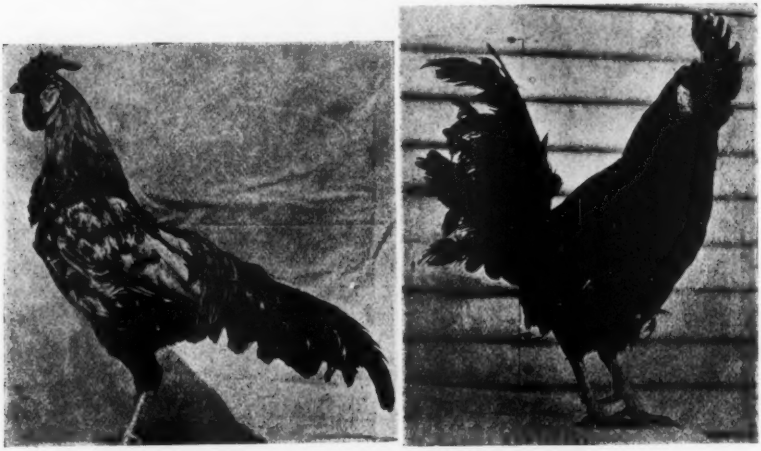
accordance with the theoretical ratio. As in the case of the males, the spangled hens showed different degrees of the extension of black and

white. In figure 14, is seen one of the spangled hens, showing some excess of white, especially on the wings, while figure 15 is one of the non-spangled hens from the mating. The latter has the characteristic coloring of the Brown Leghorn which was also present in one of the other hens.

It should also be mentioned that the recessive golden bay ground-color replaced the white in two of the spangled birds from this mating.

The analysis of the mating is as follows:

	Males	*Females*
Parents	$Z^S Z^s$	$Z^S W$
Gametes	Z^S, Z^s	Z^S, W
Offspring	$Z^S Z^S$, $Z^S Z^s$	$Z^S W$, $Z^s W$



FIGURES 16 and 17.—F_2 spangled and non-spangled cocks after cross between Hamburg female and Leghorn male (mating 5).

MATING 5. THE F_2 GENERATION FOLLOWING MATING 2

It is of interest to compare with the foregoing the F_2 generation from the original cross between the Brown Leghorn cock and the Silver Spangled Hamburg hen. Here the expectation is that both spangled and non-spangled birds should appear in equal numbers in each sex, whereas, as has been seen, the F_2 generation from the reciprocal cross lacked non-spangled males.

The mating yielded spangled and non-spangled cocks and hens in approximately equal numbers. The four types of birds are shown in figures 16 to 19.

Varying degrees of an excess of black and of white were observed, as in mating 4, and one male was produced with the spangles on a golden bay ground (figure 20).

FIGURES 18 and 19.—F_2 spangled and non-spangled hens after cross between Hamburg female and Leghorn male (mating 5).

The analysis follows:	*Males*	*Females*
Parents	$Z^S Z^s$	$Z^s W$
Gametes	Z^S, Z^s	Z^s, W
Offspring	$Z^S Z^s$, $Z^s Z^s$	$Z^S W$, $Z^s W$

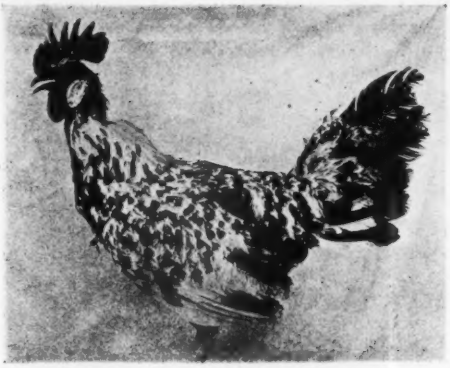
MATING 6. SELF-BLACK F_1 HENS AND COCKS FROM MATINGS 1 AND 2 WITH PURE NON-SPANGLED

It has yet to be shown that the self-black birds, appearing in the F_1 generation after the original crosses, which theoretically should be spangled but which have been interpreted as the result of a cumulative effect of multiple factors for black, completely obscuring the spangles, are, nevertheless, capable of transmitting spangles like the spangled individuals from the same matings.

FIGURE 20.—F_2 cock from same cross as figures 16 to 19, showing spangles on golden bay ground (mating 5).

Three of the black cocks have been tested by breeding them to non-spangled hens. If they carry spangling factors, they should be heterozygous for them and should, under the conditions of the test, produce both spangled and non-spangled sons and daughters just as do the F_1 spangled

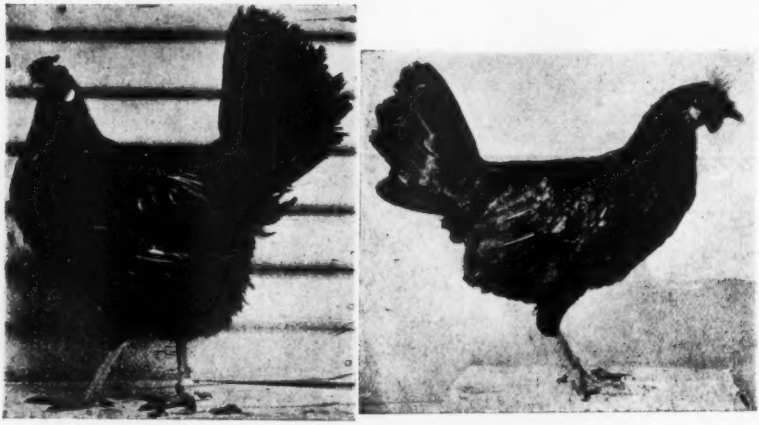
cocks under the same circumstances. This was found to be true of all of the cocks so tested, but it will suffice to illustrate the progeny of only one, as all behaved exactly alike. The cock selected for the example is



FIGURES 21 and 22.—F_2 spangled and non-spangled cocks from mating of F_1 non-spangled female and black F_1 male, carrying the spangling factor (mating 6).

the one shown in figure 6 from mating 1. He was bred to one of the non-spangled hens from mating 2 which have already been shown in mating 3 to be incapable of transmitting spangles. Spangled and non-spangled cocks and hens from this mating are reproduced in figures 21 to 24,

which should be compared with figures 16 to 19 from mating 5, in which a spangled F_1 cock was bred to a similar hen. The progenies from the two matings are identical, consisting of both spangled and non-spangled males and females. The spangles on the hen shown in figure 23 occurred on a golden bay ground, and are, therefore, not sharply contrasted in the photograph.



FIGURES 23 and 24.—F_2 spangled and non-spangled hens from same matings as figures 21 to 22 (mating 6).

The analysis is as follows:

	Males	*Females*
Parents	Z^SZ^s	Z^sW
Gametes	Z^S, Z^s	Z^s, W
Offspring	Z^SZ^s, Z^sZ^s	Z^SW, Z^sW

The two black hens from mating 1 in which spangling was expected, were similarly tested for their ability to transmit the spangling factor by breeding them to Brown Leghorn cocks. They produced, in approximately equal numbers, spangled sons and non-spangled daughters, exactly like the birds shown in figures 21 and 24, respectively. If they had not carried the factor for spangling, these black hens would have had no spangled offspring. The analysis is as follows:

	Males	*Females*
Parents	Z^sZ^s	Z^SW
Gametes	Z^s, Z^s	Z^S, W
Offspring	Z^SZ^s	Z^sW

The result, therefore, of testing these black cocks and hens, leaves no room for doubt that, although showing no spangles themselves, they transmit spangling exactly as do the F_1 spangled males and females.

As to the source of the multiple factors for black, there are two possible explanations, namely, (1) all were derived from either the Brown Leghorn or from the Hamburg stock used in the experiments; or (2) some were introduced by the Leghorns and the rest by the Hamburgs. For example, under the first supposition, if three independently segregating factors for black were present in a heterozygous condition in either the Leghorns or the Hamburgs, and, furthermore, if it should require the cumulative effect of all three of the black factors to produce a self-colored black bird, then one out of every eight individuals in the F_1 generation should be black, as the parent introducing the factors should yield eight kinds of gametes, only one of which would contain the three factors. This ratio is practically the same as the observed one, which was about seven spangled birds to one black.

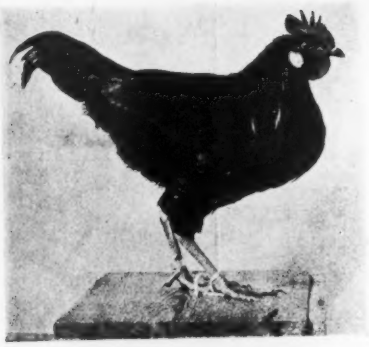
On the other hand, it would seem to be more probable that some of the factors for black were derived from the Leghorns and the rest from the Hamburgs, as no black birds have been obtained in breeding the pure stock of either the Brown Leghorns or the Hamburgs. In the absence of hybridization, then, the maximum effect could not have taken place, as one or more of the factors for black would not have been present in any zygote obtained from the cross.

MATING 7. BROWN LEGHORN FEMALE WITH F_1 MALE

The F_1 cocks have been mated with both Leghorn and Hamburg hens, giving results in entire conformity with the sex-linkage of the spangling factor. For example, in mating 7, the cock (figure 4) from mating 1 was bred to a Brown Leghorn hen, and the progeny consisted of both spangled and non-spangled males and females, according to expectation. Figures 25 to 28 show the two types in each sex. The spangled cocks were like their father, while the non-spangled hens exhibited the Leghorn coloration more or less closely. One spangled hen showed the golden bay ground-color.

The analysis is as follows:

	Males	*Females*
Parents	$Z^S Z^s$	$Z^s W$
Gametes	Z^S, Z^s	Z^s, W
Offspring	$Z^S Z^s$, $Z^s Z^s$	$Z^S W$, $Z^s W$



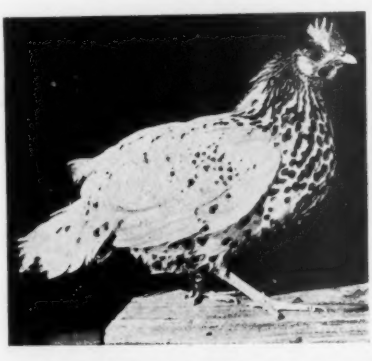
FIGURES 25 and 26.—Spangled and non-spangled cocks from back-cross of Brown Leghorn female and F_1 spangled male (mating 7).

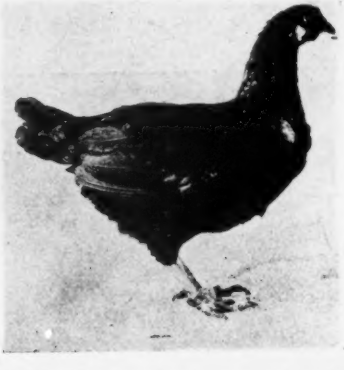
FIGURES 27 and 28.—Spangled and non-spangled hens from same back-cross as figures 25 and 26 (mating 7).

MATING 8. SILVER SPANGLED HAMBURG FEMALE WITH F_1 MALE

In this mating, a Silver Spangled Hamburg hen was bred to the F_1 cock shown in figure 7, from mating 2. Only spangled males, but both spangled

FIGURE 29.—Spangled cock from back-cross of Silver Spangled Hamburg female and F_1 spangled male (mating 8).





FIGURES 30 and 31.—Spangled and non-spangled hens from same back-cross as figure 29 (mating 8).

and non-spangled females appeared in the progeny (figures 29 to 31). Again the expectation was realized. One of the males was rumpless. Two of the hens showed the spangles on the golden bay ground.

The analysis is as follows:

	Males	*Females*
Parents	$Z^S Z^s$	$Z^S W$
Gametes	Z^S, Z^s	Z^S, W
Offspring	$Z^S Z^S$, $Z^S Z^s$	$Z^S W$, $Z^s W$

MATING 9. F_1 NON-SPANGLED FEMALE WITH SILVER SPANGLED HAMBURG MALE

In this mating the Silver Spangled Hamburg cock, used in the original cross, mating 1, was bred to a non-spangled F_1 hen from mating 2. The expectation was that all of the offspring in both sexes would be spangled. The actual results were as follows: ten spangled cocks and two black

FIGURE 32.—Spangled cock from back-cross of F_1 non-spangled female and Silver Spangled Hamburg male (mating 9).

cocks like those obtained from the original crosses; ten spangled hens and three black hens like the black hens from mating 1. One of these black males, which was identical in appearance with the black F_1 cock shown in figure 6, was bred to a non-spangled hen, with the same result as that described in mating 6, namely, the appearance of spangling in both sexes. One of the black non-spangled hens was similarly tested by mating her to a Brown Leghorn cock; her progeny consisted of four spangled sons and six non-spangled daughters.

It is clear, therefore, that these black males and females carry the spangling factor, and their presence in the brood from mating 9 is not an exception to the theoretical demand. Spangling is merely prevented from gaining expression in them by the cumulative effect of black. It may, therefore, be safely assumed that, on crossing the Silver Spangled Hamburg cock with the F_1 non-spangled hen, all of the offspring received the spangling factor.

Although four of the cocks from this mating received the factor for the black tail, the remaining six did not and showed the spangled pattern in a practically perfect form, as is seen in figure 32 which is taken from

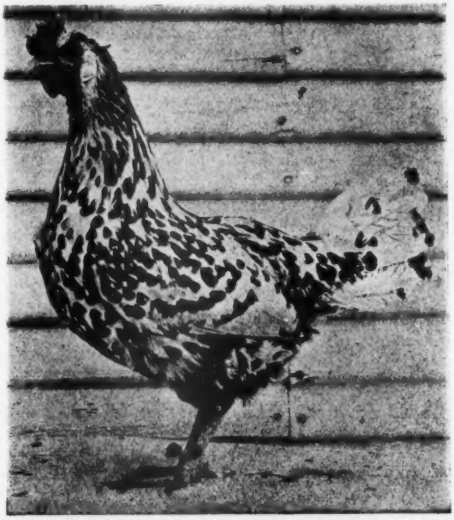


FIGURE 33.—Spangled hen from same back-cross as figure 32 (mating 9).

a photograph of one of these cocks. Of the ten spangled hens obtained, six showed the pattern in a strikingly typical manner (figure 33). Compare this figure with the photograph of the pure-bred Silver Spangled Hamburg hen shown-in figure 2, and it will be seen that the two birds are nearly indistinguishable from each other. The spangles of the pure-bred hen are perhaps somewhat larger, but there seems to be no other difference.

The analysis of the mating is as follows:

	Males	*Females*
Parents	Z^SZ^S	Z^sW
Gametes	Z^S, Z^S	Z^sW,
Offspring	Z^SZ^s	Z^SW

MATING 10. F_1 SPANGLED FEMALE WITH SILVER SPANGLED HAMBURG MALE

The same Silver Spangled Hamburg cock as was used for matings 1 and 9 was bred to an F_1 spangled female, that is, to one of his own daughters, figure 5, by a Brown Leghorn hen. Again the expectation was that all of the sons and daughters would be spangled. Only two males and three females were obtained from the mating, and they all showed the spangled pattern. The former, figure 34, were practically identical in

FIGURE 34.—Spangled cock from back-cross of F_1 spangled female and Silver Spangled Hamburg male (mating 10).

appearance with the F_1 cocks, and the latter, figure 35, with the F_1 spangled hens, or, in other words, were like their mother.

The analysis follows:

	Males	*Females*
Parents	Z^SZ^S	Z^SW
Gametes	Z^S, Z^S	Z^S, W
Offspring	Z^SZ^S	Z^SW

CONCLUSION

Several other matings were made, but, as they throw no further light on the analysis, they may be omitted from the account.

In conclusion, it may be repeated that the inheritance of the spangled pattern is controlled by a dominant factor that is typically sex-linked.

This factor is transmitted in the manner characteristic of sex-linkage in poultry, namely, the sex chromosomes of the cocks are Z Z, those of the hens Z W. A spangled Hamburg cock bred to a Brown Leghorn hen produces spangled sons and daughters, while, reciprocally, a spangled Hamburg hen bred to a Brown Leghorn cock gives spangled sons only, the daughters being non-spangled.

The expression of spangling, however, may be greatly modified, or even entirely obscured, by other factors, especially by factors for black

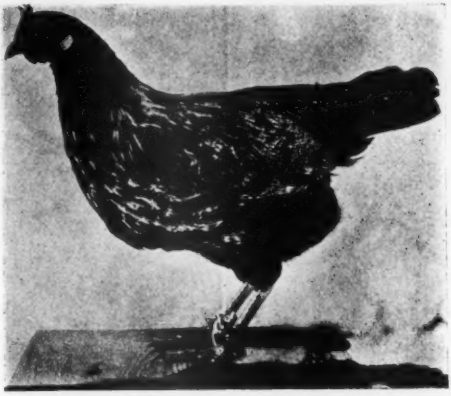


FIGURE 35.—Spangled hen from same back-cross as figure 34 (mating 10).

and by factors affecting the size and brilliancy of the spangles. It seems clear that multiple factors for black, introduced either by the Brown Leghorn or by the Hamburgs alone, or more probably, by both the Leghorns and the Hamburgs used in the experiments, are present, and that these factors give a cumulative effect, with the result that black pigmentation is developed to different degrees of extension.

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THE FACTOR FOR BITTERNESS IN THE SWEET ALMOND

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The blossoms of the almond tree are very susceptible to frost. As the almond blooms earlier than other orchard fruits it is often subjected to much more severe frosts than occur during the blooming period of the later fruits. As a result of this tenderness of the blossoms and the early period of blooming the crop secured is materially decreased. With the hope of improving our present varieties or securing new ones that would bloom late and yet be good in quality and productivity, a large number of nuts were secured from the crosses made in the first (1916) and fourth (1919) years of an almond pollination experiment being carried on by the CALIFORNIA EXPERIMENT STATION, and developed into seedlings. It was thought that if any of the important varieties, such as the Nonpareil, Ne Plus Ultra and I X L, could be made to come into blossom from five to eight days later than is normal for each variety, the hazards in almond growing would be materially decreased. If this failed, it was thought possible that some new variety could be developed. It so happens that the leading commercial varieties are the earliest bloomers and the less important varieties the latest bloomers.

The first year's planting consisted of 602 trees resulting from thirty-two crosses. Of these trees 243 came into bearing in 1922, comprising practically all the trees in twenty-one crosses. Out of the 243 trees there are 59 with bitter almonds and 208 with sweet almonds, distributed among the various crosses as shown in the table on the next page.

An examination of this table shows that there is no uniform outstanding proportion between trees possessing sweet almonds and those possessing bitter almonds in all the crosses. This would probably be expected owing to the small number of trees from each cross. However, when the totals are examined, it will be noticed that there is nearly a perfect 3 : 1 ratio, the actual ratio in terms of four being 3.028 : 0.972.

This close approximation to the theoretical Mendelian monohybrid ratio indicates that all the almond varieties represented in the above crosses are heterozygous for sweetness of the kernel. They must have the genetic constitution *Bb*, where *b* represents the factor for bitterness

as the recessive character and *B* the factor for sweetness, as the dominant character.

As far as their genetic constitution for bitterness and sweetness is concerned, it seems likely that all varieties of sweet almonds are hybrids. Many horticulturists claim that the original almond was bitter. If this is true, it is possible that a mutation occurred in the bitter almond tree with the sweet almond as the result. This probably accounts for the two general types or races of almonds existing to-day, namely, the bitter and the sweet. Such a mutation is of more than ordinary interest because

	Cross	*Sweet trees*	*Bitter trees*
Lewelling	× I X L	2	1
California Paper Shell	× Nonpareil	8	1
Ne Plus Ultra	× Drake	1	0
Ne Plus Ultra	× I X L	32	7
Nonpareil	× Péerless	5	1
California Paper Shell	× I X L	3	1
Lewelling	× Ne Plus Ultra	5	2
California Paper Shell	× Ne Plus Ultra	2	1
Reams	× Ne Plus Ultra	5	1
Nonpareil	× California Paper Shell	20	6
Reams	× California Paper Shell	7	0
Nonpareil	× Texas	12	3
Nonpareil	× Ne Plus Ultra	5	2
Drake	× Jordan	3	0
Drake	× Ne Plus Ultra	25	13
Reams	× Nonpareil	4	3
Reams	× I X L	2	2
Languedoc	× Drake	12	4
Reams	× Texas	5	3
Ne Plus Ultra	× Lewelling	10	3
Drake	× I X L	16	5
		184	59

(1) the muta t character is dominant to the normal wild type, and (2) because this mutant character consists in the loss of the bitter principle present in the wild progenitor.

It is hoped that when all the trees in the experiment come into bearing, some light will be thrown on the segregation of such factors as blossoming period, hardness of shell, quality of nut, shape of tree and leaves, and productivity. A study of these characters will no doubt add a great deal to our much needed information on fruit and nut breeding.

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